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Relaxation Folding and the Principle of the Minimum Rate of Energy Dissipation for Conformational Motions in a Viscous Medium

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Abstract—A numerical simulation of the folding of a model polymer chain of 50 units with valence bonds of a fixed length and fixed valence angle values has been performed using the strong friction approximation. The rate of energy dissipation in the system has been analyzed for conformational motions along a trajectory determined by the equations of mechanics and the trajectories characterized by random and variable deviations from the mechanical path. The validity of the principle of the minimum average rate of the energy dissipation for the conformational relaxation of a macromolecule in a viscous medium has been demonstrated. A profile of the relaxation energy funnel for the folding of a macromolecular chain has been constructed. Slow and rapid stages of folding could be distinguished in the energy funnel profile; the final state was separated from the nearest conformations of the folded chain by an energy gap.

Keywords: principle of minimum rate of energy dissipation, conformational relaxation in a viscous medium, correlation of conformational motions, energy funnel, folding dynamics

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Researchers have been working on the problem of the formation of the spatial structure of proteins and other biopolymers for more than 50 years; many brilliant ideas have been proposed during this time (see, for example, [1-10]), but the physical processes underlying this phenomenon remain incompletely understood in details [8, 10, 11]. As an example, a detailed theory that would explain the folding of protein chains into unique spatial structures on a microscopic level has not been developed yet and the roles of various structural and physical factors that affect the formation of a unique spatial structure of a given biopolymer chain have not been investigated in sufficient details. The general concept of the process of biopolymer folding implies a specific structure of the multidimensional energy landscape of the macromolecules and proteins as well. The energy landscape is assumed to form an energy funnel that determines the arrival of a representative point to a site of global energy minimum that corresponds to the native spatial structure (see, for example [2, 3, 7, 10]). The physical causes of the formation of the appropriate structure of this special multidimensional energy funnel remain unclear. The solution to this problem is of great importance for practical applications (such as protein engineering [12]). An additional problem arises because a representative point does not become entangled in numerous local and sufficiently deep minima during the

descent along the walls of the multidimensional energy funnel, but rather reaches the point of the absolute minimum of energy of the set of available configurations. This problem is also closely related to the general physical mechanisms that underlie the conformational mobility of macromolecules and the conjugation of conformational mobility and the functional activity of biopolymers (see, for example [13– 17]).

The previous study [11] was apparently the first to draw attention to the requirement for the minimum rate of energy dissipation in the case of conformational relaxation (and folding) of a macromolecular chain in a viscous medium. The condition of the minimal rate of energy dissipation upon conformational motions and conformational relaxation brings about certain dynamic effects as well. These effects are related to the emergence of collective conformational motions and the propensity of a representative point to avoid the areas of the energy landscape characterized by sharp changes in the potential energy for a limited number of degrees of freedom (the "rookie skier" principle [11]). The condition of the minimal rate of energy dissipation upon conformational motions in a viscous medium is formally similar to the ideology that underlies the Onsager principle for weakly non-equilibrium processes [18] and the concept of the minimum rate of entropy production subsequently developed by



Fig. 1. The structure of the model of a macromolecular chain. Valence bond lengths and valence angles are fixed. Rotation along the dihedral angles ϕ provides conformational mobility.

Prigogine within the framework of nonequilibrium thermodynamics [19]. Naturally, these thermodynamic principles are true for the average state of macroscopic systems. The principle of the minimal rate of energy dissipation for the conformational motions [11], in its turn, is somewhat different from the above thermodynamic concepts, since it refers to the molecular dynamics of macromolecular structures and mechanisms of formation of collective conformational motions due to the viscous resistance of the environment. These effects have not been analyzed on the microscopic level, although such an analysis would be of fundamental importance for the understanding of the physics of conformational transitions in macromolecular systems and the folding of such systems. The present study consisted of a computational analysis of the validity of the principle of the minimal rate of energy dissipation upon the conformational relaxation (folding) of a model polymer chain in a viscous medium. Other regularities of the relaxation of a system in a multidimensional space of configurations [11] are discussed as well.

Let us consider a model homopolymer chain of 50 nodes with the values of covalent bond length and valence angle fixed at 1.54 Å and 109°28', respectively (the typical values for a single carbon-carbon bond). Chain mobility is mediated by rotation of the nodes around the bonds between the adjacent nodes (Fig. 1).

The interaction of atoms that are not connected by covalent bonds (the *i*th atom and the *j*th atom, where $j = i + n, n \ge 3$) is defined by the Lennard-Jones potential, which depends on the distance r_{ij} between the nodes and the parameter u_{ij} that characterizes van der Waals interactions and is the same for all nodes

$$u_{ii} = 4\varepsilon[(\sigma/r_{ii})^{12} - (\sigma/r_{ii})^6].$$
(1)

The depth ε of the potential well is assumed to be $5 \cdot 10^{-14}$ erg (or approximately 362 K) and the van der Waals diameter of the node $\sigma = 3.43$ Å.

If the chain is stretched at the initial moment of the observation period, it will fold due to non-covalent interactions. The potential energy of the chain will thus decrease and the excess of potential energy will dissipate irreversibly due to viscous friction forces. The equations of motion for the links of the chain in a viscous medium will have the form

$$\gamma \dot{\vec{r}_i} = -\frac{\partial U}{\partial \vec{r}_i} + \vec{R}_i, \ i = 1, 2, \dots, 50.$$

In this study, we will assign a value of 10^{-6} g/s to the coefficients of friction γ in all nodes of the chain. This corresponds to the friction coefficient of a particle of 3.43 Å in diameter in the medium with a viscosity of approximately 3 P or to a particle with a diffusion coefficient of the order of $4 \cdot 10^{-8}$ cm²/s at 300 K. The absolute value of γ does not have essential importance for finding the solution of the system of equations (2) for these effects, although γ defines the time scale for the numerical simulation of the chain-folding kinetics. As shown in [20], the inertial terms can be ignored if movements with amplitudes that exceed 0.5 Å in water or another condensed medium are considered. Therefore, the respective inertial terms (the components that included acceleration) were excluded from the equation of motion (2). The potential energy of the chain is the sum of pairwise atom-atom interactions (1):

$$U = \sum_{j \ge i+3} u_{ij} = 4\varepsilon \sum_{j \ge i+3} \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^{6} \right], r_{ij} = |r_j - r_i|. (3)$$

Since the values of covalent-bond lengths and valence angles α are fixed in the chain, the respective constraint force values R_i that emerge due to mechanical conditions imposed on the system [21] are included into the equations (2):

$$r_{ij+1} = |\vec{r}_{i+1} - \vec{r}_i| = I_1 = 1.54 \text{ A},$$

$$r_{ij+2} = |\vec{r}_{i+2} - \vec{r}_i| = I_2 = I_1 \sqrt{2(1 - \cos \alpha)} = 2.515 \text{ Å}.$$
 (4)

Constraint force values can be determined using conventional methods, such as the method of undefined Lagrange multipliers [22], which yields a system of 5N - 3 (where N is the number of nodes) algebraic-differential equations:

$$\gamma \dot{\vec{r}}_{i} = -\frac{\partial U}{\partial \vec{r}_{i}} + \sum_{k} \lambda_{k} \left(\vec{r}, \dot{\vec{r}}, t \right) \frac{\partial f_{k}}{\partial \vec{r}_{i}}, \quad i = 1, 2, \dots, 50, \ k \in f,$$

$$f_{ii+1} = \left(\vec{r}_{i+1} - \vec{r}_{i} \right)^{2} - I_{1}^{2} = 0, \qquad (5)$$

$$f_{ii+2} = \left(\vec{r}_{i+2} - \vec{r}_{i} \right)^{2} - I_{2}^{2} = 0,$$

where λ_k are the Lagrange multipliers, *f* is the set of numbers of mechanical constraints imposed on the node *i*, and *r* is the set of coordinates of the nodes of the chain. The system of equations (5) should be supplemented by the initial conditions:

$$\vec{r}_i(0) = \vec{r}_i^{\ 0}.$$
 (6)

The initial coordinates of the links of the chain used in the present study correspond to a fully stretched configuration that is in a state of unstable equilibrium and cannot undergo relaxation in accordance with the equations (2). Therefore, the relaxation was initiated by little shifting of the positions of nodes 1 and 50 of the chain from the positions that corresponded to the unstable equilibrium.

System (2) was different from the Langevin equations used in [11], since rigid constraints (4) were imposed on the nodes of the chain and thermal noise was eliminated. The latter condition means that a purely relaxational process was considered and the system would not be able to exit a local energy minimum for the coordinates of all nodes (if the system would enter such a minimum during relaxation) due to the absence of thermal fluctuations. Therefore, the energy depth of the relaxation process served as a test for the smoothness of the relaxation energy funnel and the validity of the "rookie skier" principle [11]. This principle consists in the propensity of the representative point to pass around the areas that correspond to sharp changes of potential energy along a relatively small number of coordinates of the configuration space due to the multidimensional character of the energy landscape [11].

Let us also mention several mathematical relationships for the system of equations (2). These relationships are almost identical to those reported in [11]. Multiplication of (2) by the velocity of the *i*th link and summation for all particles will yield the following relationship (since the work of the constraint forces is zero) [21]:

$$S = \gamma \sum_{i} \left(\dot{\vec{r}}_{i} \right)^{2} = -\frac{dU}{dt}.$$
 (7)

Since the sum of the forces that act inside the system is zero, summation of the equations (2) yields:

$$\sum_{i} \dot{\vec{r}}_{i} = 0.$$
 (8)

The equation (8) means that the geometric center of the system (that is identical to the center of mass in this case) remains immobile if all friction coefficients are equal.

Vector multiplication of the equations (2) by the radius vector of the node *i* and summation for all nodes (with the zero value of the sum of moments of all internal forces taken into account) yields:

$$\sum_{i} \left[\vec{r}_{i} \times \vec{r}_{i} \right] = 0, \tag{9}$$

that is, the sum of the moments of the friction forces is zero. In other words, the system will not acquire torque during the relaxation. The relations (8) and (9) are of a general character [11] and can be used to verify the accuracy of the computational procedures.

The relation (7) connects the power (rate) of energy dissipation and the rate of decrease of the potential energy of the system. These values must be

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equal; therefore, the decrease of potential energy over the time T can be expressed by the formula

$$U(0) - U(T) = \int_{0}^{T} S(t) dt.$$
 (10)

The principle of the minimum energy-dissipation rate for the conformational motions of a macromolecule in a viscous medium [11] discussed here implies that the *S* value in (7) will be minimal upon movement along a mechanical path that is the solution of equations (2) or (5) (of all alternative r(t) paths). The condition for a minimum that can be obtained by varying *S* is the following

$$\delta S = \gamma \sum_{i} \left[\left(\delta \vec{r}_{i} \right)^{2} + 2 \vec{r}_{i} \delta \vec{r}_{i} \right] \ge 0.$$
 (11)

The value of rate variation depends on the deviation of the velocity of a given node at a given time from the velocity of this node defined by the mechanics equations (2) or (5). If the deviation of the node velocity from the predefined value on the trajectory is considered as a random value with a mean of zero at each time point, one can calculate the average value of δS and automatically achieve a situation for which the principle of the minimum rate of energy dissipation is valid. Assessment of the average value of the variation of the dissipation rate (or the average value of the deviation of the energy-dissipation rate from the dissipation value determined by the equations of mechanics) along the entire mechanical trajectory of chain folding is usefull for the analysis of the validity of the principle of the minimum rate of energy dissipation during the folding of a macromolecular chain in a viscous medium, as well as the assessment of the dependence of the above named value on the parameter that defines the scale of deviation of the perturbed trajectories from the trajectory determined by the equations (2) or (5):

$$\langle \delta S \rangle = \frac{1}{T} \int_{0}^{T} \delta S(t) dt, \qquad (12)$$

where T is the length of the trajectory of chain folding.

Let us now consider the numerical solution of equations (5). The solution was obtained using a newly developed procedure based on the modified Euler's method [23] for numerical solution of differential-algebraic equations. The local error of the procedure was of the order Δ^3 , where Δ is the step of integration over time for the system (10⁻² ns in the present study).

Assessment of the accuracy of the procedure showed that the geometric center of the chain shifted by approximately $5 \cdot 10^{-14}$ Å during approximately 450 ns; in other words, the sum of velocities (8) was on the order of $0.5 \cdot 10^{-14}$ Å/ns. The value of the total



Fig. 2. The dependence of potential energy (1) and the work of the friction forces (2) on time during the process of chain folding. The dependences are slightly shifted along the vertical axis for the sake of clarity, since the respective energy changes are virtually identical.

moment (9) was on the order of 10^{-7} Å²/ns; therefore, the system as a whole is expected to make less (and even much less) than 10^{-5} of a full turn during the time used in the calculations. These tests demonstrate the correctness of the algorithm and the good accuracy of the computational experiment.

The comparison of the decrease of the potential energy of the system and the work of friction forces is illustrated by Fig. 2.

The deviation of the work of friction forces from the decrease of the potential energy averaged by time (10) is approximately $5 \cdot 10^{-17}$ erg and the relative accuracy of the coincidence of the curves is on the order of 10^{-6} . The overall decrease of the potential energy of the system is approximately $850 \cdot 10^{-14}$ erg, or the energy gain of 170 full-fledged pairwise contacts of the nodes of the chain, which corresponds to the formation of a dense globule.

The profile of the relaxation energy funnel for the folding of the system is visualized in Fig. 3; the profile was constructed by mirroring the curve in Fig. 2 relative to the vertical axis that passed through the lowest point.

The stepwise character of chain folding is apparent from Figs. 2 and 3, and especially from Fig. 4. The relatively slow initial (pre-folding) stage accounts for approximately 60% of the time and is characterized by an approximately 30% decrease in energy. The initial stage and later more faster stages accompanied by periods of temporary relative stabilization of the chain energy. The steepness of the energy decrease curve tends to increase during the later stages of the process, whereas the energy amplitude of these stages progres-



Fig. 3. An energy funnel for the relaxation folding of a chain.

sively decreases. The final stage of energy reduction is the fastest, with an approximately 5% decrease of the total energy value that takes approximately 5% of the entire folding time. Interestingly, the resulting pattern is very similar to that implied by the current concept of protein folding [2, 3], which also includes the existence of an energy gap near the final (native) state [6, 10]. However, one should remember that the model does not provide a faithful reproduction of the actual structure of a polypeptide chain and the comparison of the model to the processes that occur in actual biopolymer systems is beyond the scope of the present study.

The stepwise character of chain folding is apparent from the temporal pattern of the energy-dissipation rate S (Fig. 4).

The contributions of the terminal atoms of the chain to the energy-dissipation rate are shown in Fig. 5 for comparison (one should note that the value of the dissipation rate divided by the number of nodes is shown in Fig. 4).

The points of the time axis that correspond to strong bursts of the energy dissipation rate are marked in Figs. 4 and 5. The correlation between strong bursts of the energy dissipation rate and quick movement of the terminal links of the chain associated with considerable structural remodeling of the entire chain is apparent. The comparison of the ratio of the general dissipation rate and the rate values in Fig. 5 shows that a burst of the dissipation rate near 70 ns is largely due to the movement of the terminal nodes only, since the motions of these groups do not have to be correlated with the movements associated with other nodes.



Fig. 4. The change of the specific rate of energy dissipation upon chain folding. Time points that correspond to flashes of the energy dissipation rate are marked on the axis (compare to Fig. 5).



Fig. 5. The contributions of the terminal atoms of the chain to the energy dissipation rate. Time points that correspond to bursts of the specific dissipation energy rate are marked on the axis.

However, movement of the terminal groups alone cannot account for the full power of the burst in the intensity of energy dissipation around the time point of 350 ns, but rather makes an approximately 20% contribution to the overall effect. This observation is in agreement with our earlier findings [24] and the propensity of chain folding to start from the terminal groups that can rotate without the requirement for correlations with the rotation around the bonds located at a distance from the ends of the chain (in the case of a completely stretched initial configuration of the chain), whereas the minimal rate of energy dissipation in a macromolecule of a globular configuration requires a tighter consistency between the turns around all chains.

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A typical pattern for the correlation of movements along the conformational degrees of freedom is shown in Fig. 6 (the pattern corresponds to the time point of approximately 40 ns). The direction of the turns around the bonds apparently varies, so that resistance of the medium can be minimized.

Let us now consider the implementation of the principle of the minimum rate of energy dissipation (7) for the conformational rearrangements of macromolecules in a viscous medium. For this, we will consider the variation δS of the rate of energy dissipation upon the deviation of the relaxation trajectory from that defined by the equations of mechanics (2) or (5). Deviations from the mechanical path will be simulated as follows: a perturbation represented by a random-



Fig. 6. A typical instant distribution (at time \sim 40 ns) of the velocity of angular rotation around the valence bonds as dependent on the number of the bond in the chain. The directions of rotation alternate; thus, the energy dissipation rate is minimized.

angle rotation around each bond will be introduced at every 100th step of integration of the equations of mechanics (5). The rotation angle ϕ follows a normal distribution with a mean value of zero and the standard deviation σ (not to be confused with the symbol in the formula (3)):

$$p(\phi) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{\phi^2}{2\sigma^2}\right).$$
 (13)

Independent sets of random-angle turns around every bond with a distribution conforming to (13) were used in each test. The number of tests for turns around the angles was 100 for each point of the mechanical trajectory at a predefined σ value. Selection of the results of the statistical tests was performed to avoid very large changes in spatial positions of chain nodes, since these changes are devoid of physical meaning. Such changes can occur upon rotation around the bonds that are close to the center of the chain, even at small rotation angles. The selection was based on the Metropolis criterion [25] formulated as:

$$M = M_0 \exp\left[-\sum_i \left(\delta \vec{r}_i\right)^2 / Nb^2\right], \qquad (14)$$

where *M* is the probability of the acceptance of the result of the test, N = 50 is the number of nodes in the chain, δr_i is the displacement of node *i* in a specific test, b = 0.5 Å is the threshold value for node displacement, and M_0 is the normalization multiplier that depended on the set of 100 test results in each point. As a result, perturbed configurations were obtained after every 100 steps of integration of the mechanical equations for the relaxation of the initial conformation of the chain. The configurations were used as the starting conditions for solving the equations of mechanics (5). These equations were further integrated to simulate the area of the perturbed trajectory within the 100-step



Fig. 7. The dependence of the variation of the energy dissipation rate on the deviation of the trajectory of chain folding from the mechanical trajectory due to random rotations around the bonds with a mean value of zero and a standard deviation of 1° .

interval; the procedure of perturbation of the mechanical trajectory was then repeated. The value of the variation of the rate of energy dissipation (11) was calculated at each time point. The structure of these variations is characterized by rapid oscillations; therefore averaging over two variation intervals (that is, 200 neighboring points) was performed. Analysis of the set of results obtained for the variation of energydissipation rate at the values of the parameter σ ranging from 0.01 to 5° revealed the following: the $\delta S(t)$ dependence was close to zero at σ values lower than $\sim 0.1^{\circ}$, with the exception of the areas characterized by sharp bursts of S(t). These areas (points) correspond to a somewhat rapid downward movement of the representative point along a steep slope of the energy funnel. Rapid stochastic variation of the energy dissipation rate is characteristic of these areas and the average value of the rate was is close to zero. The oscillation amplitudes are correlated to the value of the energy dissipation rate, but the former are six to eight orders of magnitude lower than the latter. A tenfold increase of σ (to 1°) resulted in an increase of the amplitude of $\delta S(t)$ oscillation in the areas of high energy dissipation rates. An increase by approximately three orders of magnitude was observed (Fig. 7), but the value still remained lower than S(t) itself by approximately three orders of magnitude. A characteristic plateau at time points that correspond to the folded conformation of the chain became apparent when σ exceeded 0.5°. The average values of $\delta S(t)$ within the intervals limited by two consecutive perturbations were positive in all cases, this being indicative of the validity of the principle of the minimum average rate of energy dissipation along the trajectory determined by the equations of mechanics (5).



Fig. 8. The dependence of the average variation of the energy dissipation rate on the standard deviation value for the distribution function of the angles of rotation around the bonds (a characteristic of the perturbation of the mechanical trajectory) in increments of 0.5° .

The dependence of the mean value of the variation of the energy dissipation rate on the intensity σ of random perturbations of the trajectory along the entire trajectory of chain folding (12) is illustrated by Fig. 8. The variation of the energy dissipation rate is apparently very close to zero in the case of slight variations of the trajectory, as should be expected given the necessary conditions for an extremum of the functional (7). The increase of the perturbation scale for the mechanical trajectory yields positive values for variation of the dissipation rate; this is indicative of the existence of a minimum of this functional on the mechanical trajectory.

Let us consider the physical meaning of the effects of an increase of the variation of energy dissipation rate in the areas associated with sharp acceleration of the relaxation process and an area associated with an equilibrium configuration of a folded chain. As follows from the equation (7), the $\delta S(t)$ value is proportional to the variation of the rate of change of the potential energy. Three situations that take place as the trajectory is varied at points that correspond to different values of the rate of potential energy change in the system are illustrated in Fig. 9. A random small increment of the coordinates transports the representative point into the close vicinity of a certain point that has some position that corresponds to the mechanical trajectory. If the initial point is located in an area characterized by a smooth change of the potential energy surface (points 1 and 3 in Fig. 9), the variation of the relaxation rate determined by the change in the potential gradient will be slight and weakly sensitive to the direction of movement of the representative point. The change in the relaxation rate (and thus in the rate of energy dissipation) in an area characterized by a



Fig. 9. The interpretation of the time dependence of the variation of the energy dissipation rate (Fig. 7). Random variation of the trajectory (location of the representative point) in the vicinity of points 1 and 3 on the potential energy surface does not have an apparent effect on the relaxation rate, regardless of the direction of variation. The relaxation rate depends on the direction of variation if the trajectory is varied in the vicinity of point 2. Any variation of the location at the point 4 that corresponds to the minimum of the potential energy will lead to an increase in the relaxation rate and thus to an increase in the dissipation rate.

sharp change in the profile of the energy landscape (a steep gradient of potential energy) will be rather high and strongly dependent on the direction of the change of the location of the point in the multidimensional configuration space (point 2 in Fig. 9). This phenomenon is observed in the time range of 300-350 ns in Figs. 2, 4, and 7. Finally, any variation of the position of a point relative to the minimum of the energy surface will increase the potential gradient and the rate of energy dissipation at the final stage of the folding process (point 4 in Fig. 9). It is observed at the time of approximately 400 ns (Fig. 7).

Thus, the results of a numerical simulation of the relaxation folding process are in agreement with the general physical concept of the dynamics of the process formulated in [11] and satisfy the certain conditions that define the regime of the conformational changes related to rotation around bonds and the folding of macromolecular chains in a viscous medium. Rotational movements around the bonds at every time point occur in different directions but occur in a way that provides the minimal average rate of energy dissipation. This is similar to the well-known principle of non-equilibrium thermodynamics [18, 19], but that principle was shown to be applicable to the microscopic process of conformational motion in the present study as well. The time course of relaxation folding

of the chain is non-uniform, with periods of relatively rapid structural changes alternating with periods of relatively slow conformational adjustment of the structure to new conformational states that open up new opportunities for relatively rapid and large-scale changes of the conformation of the macromolecule. The relaxation profile of the potential energy that illustrates the kinetic stages of energy decrease during the folding of the model chain is in good agreement with the existing general concepts of the shape of the energy funnel for the folding of protein structures [2-4].

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REFERENCES

- 1. C. Levinthal, J. Chem. Phys. 65, 44 (1968).
- 2. P. G. Wolynes, Phil. Trans. R. Soc. Lond. **363**, 453 (2005).
- J. N. Onuchic and P. G. Wolynes, Curr. Opin. Struct. Biol. 14, 70 (2004).
- E. R. Henry, R. B. Best, and W. A. Eaton, Proc. Natl. Acad. Sci. USA 110, 17880 (2013).
- J. Kubelka, T. K. Chiu, D. R. Davies, et al., J. Mol. Biol. 359, 546 (2006).
- 6. E. I. Shakhnovich and A. M. Gutin, Nature **346**, 773 (1990).
- 7. A. V. Finkelstein and O. B. Ptitsyn, *Protein Physics* (Universitet, Moscow, 2002; Academic Press, New York, 2002).

- K. A. Dill and J. L. MacCallum, Science 338, 1042 (2012).
- 9. R. Lindorff-Larsen, S. Piana, R. O. Dror, and D. E. Shaw, Science **334**, 517 (2011).
- 10. E. Shakhnovich, Chem. Rev. 106, 1559 (2006).
- 11. K. V. Shaitan, Biophysics (Moscow) 60, 692 (2015).
- D. A. Dolgikh, O. B. Ptitsyn, A. N. Fedorov, et al., Mol. Biol. (Moscow) 26, 1242 (1992).
- K. V. Shaitan and A. B. Rubin, Mol. Biol. (Moscow) 14, 1323 (1980).
- K. V. Shaitan and A. B. Rubin, Mol. Biol. (Moscow) 15, 368 (1981).
- 15. K. V. Shaitan, Biofizika 9, 949 (1994).
- K. V. Shaitan, in *Stochastic Dynamics of Reacting Bio-molecules*, Ed. by W. Ebeling, L. Schimansky-Gefer, and Y. M. Romanovsky (World Scientific, Singapore, 2003), pp. 283–308.
- 17. A. B. Rubin, *Biophysics*, Vol. 1: *Theoretical Biophysics* (Inst. Comput. Res., Izhevsk, 2013) [in Russian].
- 18. L. Onsager, Phys. Rev. 38, 2265 (1931).
- I. Prigogine, Introduction to Thermodynamics of Irreversible Processes, 3rd ed. (Wiley Interscience, New York, 1968; Research Center "Regular and Stochastic Dynamics," Izhevsk, 2001).
- K. V. Shaitan and S. S. Saraikin, Biophysics (Moscow) 45, 397 (2000).
- S. M. Targ, A Concise Course in Theoretical Mechanics, 10th ed. (Vysshaya Shkola, Moscow, 1986) [in Russian].
- 22. D. Frenkel and B. Smit, Understanding Molecular Simulation. From Algorithms to Applications (Academic Press, New York, 2002).
- N. S. Bakhvalov, N. P. Zhidkov, and G. M. Kobel'kov, Numerical Methods (Binom, Moscow, 2011) [in Russian].
- 24. K. V. Shaitan and I. V. Fedik, Biophysics (Moscow) **60**, 335 (2015).
- 25. N. Metropolis and S. Ulam, J. Am. Stat. Assoc. 44, 335 (1949).

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