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SOME PROBLEMS OF CLUSTER DYNAMICS: MODELS OF MOLECULAR SCISSORS

Yu.M. Romanovsky, A.V. Netrebko

The problem of Brownian motion of dumbbell-shaped particles A-B that can fall apart is considered and solved using of the methods of molecular dynamics. The problem of «cutting» of the positively charged acetylcholine A-B with the formation of parts A and B in the active site of the hydrolytic enzyme acetylcholinesterase is considered as an example. The models are based on the data files from Protein Data Bank. The conditions of diffusion limitation of AChE reaction rate related with the entrance of the A-B molecules and the escape of the molecules A and B from the pocket of the active site are determined.

As a rule, enzyme-substrate interaction is reduced to loosening or breaking of a certain bond in the substrate molecule. Thus, any two-atom molecule A-B or A-A can be considered as a model of the substrate. The problem of interaction of the simplest substrate with the enzyme can be formulated as the problem of A-B molecule in the field of several ligands.

M.V. Volkenstein, I.B. Golovanov, V.M. Sobolev [1]

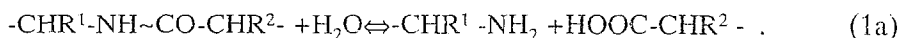
The reaction of breaking of the chemical bond in a molecule A-B (not necessarily two-atomic) can take place in liquid phase when the molecules collide with the solvent molecules. But the probability of this process is low. Let us explain it using as the examples the breaking of the peptide bond in a protein molecule and of the ester bond in the neuromediator acetylcholine (ACh) in water at room temperature. After that we consider an effective «cutting» of these bonds by the molecular scissors (hydrolytic enzymes).

During digestion peptide bond must be broken, and the protein chains splitted down to amino acids get into blood and are brought by the blood flow to all cells of the living organism. The «cutting» of the ester bond in acetylcholine molecule with the formation of choline and acetate must take place continuously in the intercell space of the neurons for maintaining of the normal functioning of the nerves.

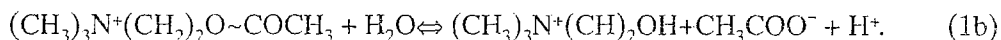
The main aim of this work is formulation and solution of specific problems of nonlinear dynamics that are born by the consideration of the mechanism of action of the molecular scissors. We try to elucidate the physical essence of some stages of the catalytic act.

1. Breaking of the peptide and ester bonds without enzyme

The reaction of breaking of the peptide bond can take place in aqueous solution of the protein. However this is a very slow process. It takes place as follows:



Here R^1, R^2 are the residues of the two neighbor amino acids, the peptide bond between which (wave line) must be broken. The reaction of hydrolysis of ACh with the formation of choline (XOH) and acetate (CH_3COO^-) is quite similar:



The rate of breaking of the bond V is proportional to the substrate concentration $[S]$ (protein or ACh) and the concentration of the $[\text{OH}^-]$ ions, (and, possibly, $[\text{H}_3\text{O}^+]$; while $[\text{OH}^-]=[\text{H}_3\text{O}^+]$), that work as catalyses in aqueous solution:

$$V \sim (kT/\hbar) (1/n) \exp(-E/kT) [S] [\text{OH}^-] = K [S] [\text{OH}^-] \quad (2)$$

Factor (kT/\hbar) determines the mean rate of the attacks of the peptide bond by the hydroxyl ions; k and \hbar are Boltzmann and Planck constants. The factor $1/n$ is a so-called steric factor that takes into account the fraction of the successful attacks that can result in bond breaking. The value E in the exponential is the energy of the valent bond (see, for example, [2]).

Assume that

$$1/n = \exp(ST/kT),$$

where $S=-k \ln(n)$. Then the value of the absolute rate K can be represented as

$$K = (kT/\hbar) \exp(-F/kT), \quad (3)$$

where $F=E-TS$ is the free energy.

It is seen from the formula (2) that the rate limit is $kT/\hbar \sim 0.6 \times 10^{13}$ Hz. For a very effective enzyme (e.g., acetylcholinesterase) this value is about 10^8 Hz, whereas for the reaction in water it is lower by several orders of magnitude. The action of $[\text{OH}^-]$ results in the decrease of the value of E . Otherwise, the factor $\exp(-E/kT)$ was very small: about $100 kT$ for the valent bond.

2. How does the enzyme influence the rate of breaking of the chemical bond of the substrate molecule?

What happens if an enzyme works as catalyser instead of $[\text{OH}^-]$ group? Its function is reduced to the change of the entropy factor $1/n = \exp[T\Delta S/kT]$ and to lowering of the energy barrier ΔE . Now other energy sources except thermal fluctuations and the sorbtion energy are necessary for the bond breaking.

Sometimes the large and complex enzyme molecule is treated as a «structured solvent» for the substrate that can form an enzyme-substrate complex with the substrate molecule. Going back to the epigraph one can say that the molecule A-B gets into a specially organized force field and is fixed therein. The parameters of the potential relief are fluctuating all the time. Some oscillating atoms or groups of atoms of the enzyme active site play the role of the hydroxyl group and the potential relief inside the active site is organized in such a way that the effective value of the breaking energy gets lower. In other words, the protein molecule is a specialized molecular machine (molecular scissors) and the substrate molecule is an item being processed by this machine.

Our task is to formulate the mathematical models of individual stages of the catalytic act in order to estimate the time necessary for each of the stages. In principle, the dynamic model of the enzyme-substrate interaction in aqueous solution consisting of thousands of nonlinear differential equations can be solved by means of the methods of molecular dynamics at the supercomputer. It means that one can trace the realizations of the following random processes:

- drift of the substrate molecule (SM) towards the entrance cleft of the active site (AS) of the enzyme;
- penetration of the SM into the fluctuating cleft of the AS;
- contact of the SM with the catalytic group (CG) inside the AS and complementary binding of the SM;
- reconstruction of the CG and the attack of the SM bond by the active atom (ion) of the CG (this is the act of «cutting» of the SM!);
- escape of the reaction products A and B after breaking of the bond in the A-B molecule.

At all the stages it is important to estimate the statistic parameters of vibrations of the atomic groups that form the entrance cleft of the AS and vibrations of SM relative to the active atom of the CG. Our aim is quite modest: we plan to develop a series of «cluster» models of the enumerated individual stages each of which contains a minimal number of the variables. This will allow us to understand better the physico-chemical mechanisms of the individual intermediate stages of the catalytic act. Besides that, such models make it possible to pose new problems of the Brownian motion. Specifically, the problems of motion of the interacting Brownian particles of the varying shape in the potential 2D (or 3D) relief with several minimums the parameters of which are fluctuating.

In our previous considerations [3 - 13] we used as an example alpha-chymotrypsin (ACT) molecule. In this paper we describe some individual stages of the catalytic act using as an example another remarkable enzyme - acetylcholinesterase (AChE).

3. Acetylcholinesterase

Enzyme AChE (as well as ACT) is related to the class of the serine proteases. It catalyses the reaction of splitting of the neuromediator ACh ($M=146$) to choline ($M=59$) and acetate ($M=104$) (see [14]).

Note that AChE has a positive charge and the reaction products have different charges. The shape of the AChE molecule resembles a dumbbell.

ACh is used for «communication» of the neurons and for chemical control of the «executing» cells, e.g., muscle ones. It concentrates in the synaptic bubbles that are localized in the synapses. The size of the synapse is about 1 micron and it lies close to the target-cell. When the electric pulse of action that is generated in the neuron body reaches the synapse via the dendrite (active cable), the release of the ACh molecules into the intercellular space (synaptic cleft with the width of 50-100 Å) is induced. One pulse with the duration of about 1 ms provides the release of several dozens of ACh molecules. The diffusion brings these molecules to the membrane of the target cell where the receptors of the neuromediators are located. After making contact with the receptor ACh induces the change of the ionic conductivity of the membrane. After activation of several receptors the electrically excited target-cell can generate its own pulse of action that, in turn, propagates along the nerves, etc. This remarkable mechanism is the basis of communication of the majority of brain cells [15].

It is evident that if the excess of the ACh molecules is not removed quickly enough from the intercell space after activation of the receptors then the functioning of the brain

is paralyzed. To avoid this the biological evolution «invented» a special effectively working (like scissors) enzyme - acetylcholinesterase.

AChE molecules ($M \approx 260\ 000$) are attached to the outer side of the synaptic membranes. Under normal regime one AChE molecule cuts one ACh molecule in $100\ \mu\text{s}$. This reaction is 100 times faster than the reaction of the same type with hydrolytic enzymes (e.g., ACT). The reaction products go back to the synapses and are used for the synthesis of the new neuromediator molecules.

High activity of the enzyme is attained due to organization of the active site. The catalytic apparatus is similar to that of ACT. The catalytic triad $\text{Glu}^{327} - \text{His}^{440} - \text{Ser}^{200}$ is located on the bottom of a deep narrow well of the depth $21\ \text{\AA}$ and mean width of $7\ \text{\AA}$. In Fig. 1 we show the position of the ACh (substrate) molecule relative to the atoms O (Ser) and N (His). For the successful attack of the ACh bond (wave line) the oxygen atom must acquire negative charge. This is achieved (like in the analogous situation of breaking of the peptide bond in the enzyme-substrate complex of ACT) due to the proton transfer from O(Ser) to N(His) or, maybe, due to temporal binding of the proton with the substrate molecule [16].

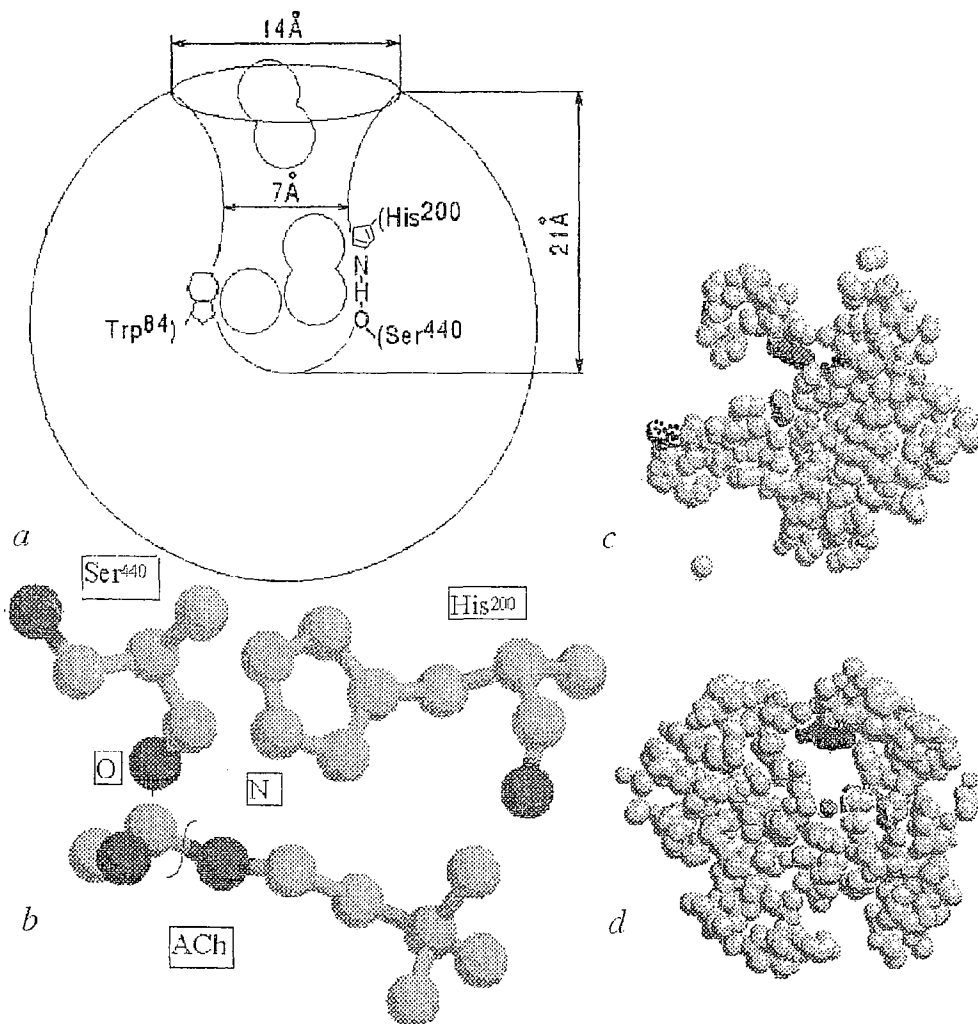


Fig. 1. *a* - Scheme of AChE AS. A dumbbell is ACh molecule in the vicinity of the catalytic triad; *b* - ACh position relative to Ser⁴⁴⁰ и His²⁰⁰. Wave line shows the peptide bond being broken; *c* - 5-Å layer cut from AChE molecule along its axis; *d* - transverse 5-Å layer cut from AChE molecule in the vicinity of the entrance

The distribution of charges at the walls of the well is such that the electrostatic field forms a cone that concentrates the substrate near the entrance and pulls inside the positively charged ACh molecules. AChE molecule has a large dipole moment ($\cong 1500$ D [14]). One of the hypotheses accounts for the removal of the reaction products in a way as follows. There is a «back door» (Trp⁸⁴) in a well wall. This «door» rotates around the C-C bond after «cutting» of the ACh molecule. The opening of this door results in the removal of the reaction products, after that the «back door» closes and at the same time the next ACh molecule falls down and binds in the AS [17].

4. How do ACh molecules get inside the AChE AS?

It was proved earlier [18, 19] that an excessive amount of ACh molecules concentrate in the area close to the entrance to the AS pocket under the action of the dipole field. It happens so because the rate of the substrate migration towards the entrance is one and a half - two times higher than the rate of AChE functioning. In connection with this we considered the problem of passing of a dumbbell-shaped particle (two plane disks of the radius r_0 , the centers of which are at the distance of $2r_0$) through a corridor formed by two Lennard - Jones potentials of the characteristic radius R_p ; the centers of the potentials are at the distance of d' (Fig. 2). The area around the origin was surrounded by an elastic wall of the elliptical shape with the center in the origin. It was assumed that a center that attracts one of the disks of the dumbbell and repulses another is located near the upper focus of the ellipse ($x_p=0; y_p=20$). In the practical computations the repulsing force was assumed to be two times weaker than the attracting one. The attracting center was simulated by means of either the potential

$$U_p = k_1((x-x_p)^2 + (y-y_p)^2)$$

or Coulomb potential

$$U_p = k_2/((x-x_p)^2 + (y-y_p)^2)^{1/2}.$$

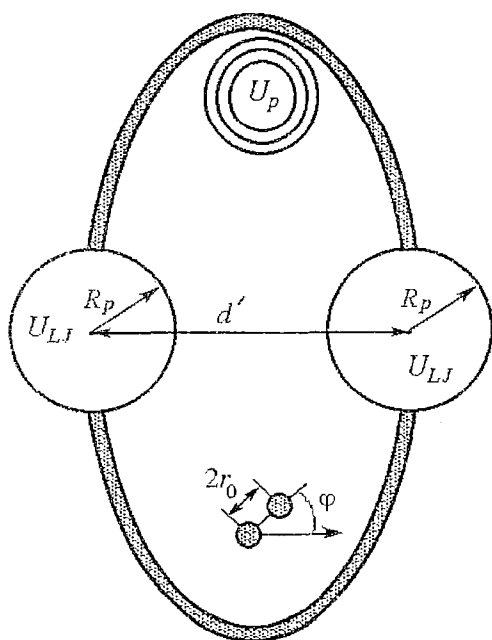


Fig. 2. Billiard with elliptical walls

The relationship between k_1 и k_2 was chosen on the basis of equality of forces acting on the particle when it goes through the gates for different types of the pulling potentials.

In the initial moment of time there are n ($n=1-20$) randomly distributed particles in the lower area. It was assumed that the dumbbells can interact with each other and with the wall according to the rules of the elastic collision. If any of the particles passes through the potential cleft (its center of gravity crosses the X -axis) then at the same time a new particle appears in the lower area (the process goes on under constant concentration of the particles in the accumulating area). We considered the mean operation rate of such a machine (the rate of penetration of the dumbbells into the upper part) as dependent on the concentration of the particles in the accumulating area, the type of the attracting force, possible external random actions, and the frequency of the possible oscillations of the potential cleft size.

The system of equations for the motion of a single dumbbell in the potential field U is as follows:

$$\begin{aligned} d^2x/dt^2 &= -\partial U/\partial x - hdx/dt + (D_a h)^{1/2} \xi_1(t) \\ d^2y/dt^2 &= -\partial U/\partial y - hdy/dt + (D_a h)^{1/2} \xi_2(t), \\ I d^2\varphi/dt^2 &= \mathbf{r}_0 \times (-\text{grad}U_1 + \text{grad}U_2). \end{aligned} \quad (4)$$

Here x and y are the coordinates of the dumbbell center of gravity; t is time; h is the friction coefficient; D_a is the amplitude of the noise action; ξ_1 and ξ_2 are delta-correlated noises (random value uniformly distributed at the interval $[-1; 1]$); I is the moment of inertia of the dumbbell; φ is the angle between the dumbbell axis and X axis, and U is the total potential of the forces acting on the center of gravity of the dumbbell:

$$U = U_{LJ1} + U_{LJ2} + U_p + U_{cont}, \quad (5)$$

U_1, U_2 are the integral potentials of the forces acting on the centers of the first and the second disks of the dumbbell (they are determined according to a similar formula); U_{LJ1}, U_{LJ2} are Lennard - Jones potentials that imitate the gates of the active site; U_p is the integral potential of the forces acting on the dumbbell from the active site; U_{cont} is the potential of dumbbells interactions with each other and with the border that surrounds the accumulating area (it was assumed in computations that the dumbbells interact with each other and with the border according to the law of elastic interaction).

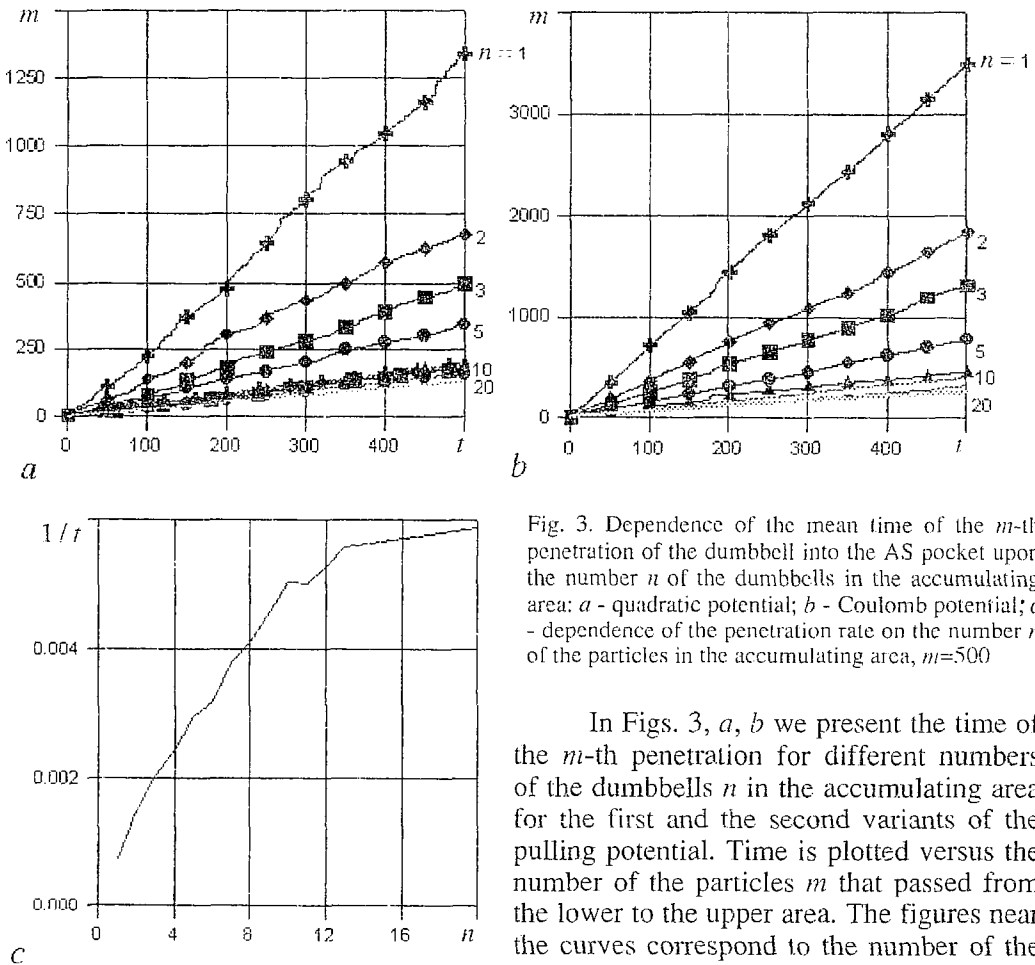


Fig. 3. Dependence of the mean time of the m -th penetration of the dumbbell into the AS pocket upon the number n of the dumbbells in the accumulating area: a - quadratic potential; b - Coulomb potential; c - dependence of the penetration rate on the number n of the particles in the accumulating area, $m=500$

In Figs. 3, a, b we present the time of the m -th penetration for different numbers of the dumbbells n in the accumulating area for the first and the second variants of the pulling potential. Time is plotted versus the number of the particles m that passed from the lower to the upper area. The figures near the curves correspond to the number of the

particles in the accumulating area n . All the dependences $m(t)$ are virtually linear, i.e. the rate of entering is almost constant and depends only on n . In course of computations for some of the dumbbells the calculated time could be larger than the theoretical one by an order of magnitude (the theoretical time is the time necessary for a particle to reach the active site from the starting point under the action of the only potential U_p). It happens so because the gates are often blocked (2-3 particles approached the gates simultaneously and could not pass through them, the neighbor particles did not allow reflection), besides that, the width of the AS gates is such that the dumbbell can pass through them if φ is close to 90° . For $n < 10$ the penetration rate increases with the increase of n and for $n > 10$ it virtually does not depend on n . If the passing time for each particle is related to the theoretical time for a single particle in the corresponding potential, the calculated curves for the cases a and b coincide. Thus, the entering rate is constant and does not depend virtually on the number of the particles in the accumulating area for $n > 10$ and depends only on the value of the pulling force.

The results slightly depend on the intensity of the noise D_a and are determined mainly by the interaction of the dumbbells. If the centers of the gate potentials oscillate harmonically with the frequency ω , then the widening of the gates results naturally in the decrease of the time t mainly due to the possible simultaneous penetration of two particles (see also [20]). However, we could not establish any correlation between the penetration rate and the frequency ω . We performed the calculations with regard to the charges of the dumbbells by means of introducing of the sum of Lennard - Jones and Coulomb potentials instead of U_{cont} . However, we did not find any substantial differences from the described results.

5. Kramers problem for 2D potential relief

We meet the problem of the particle escape from the minimum of the 2D potential relief each time when it is necessary to estimate the time of transition of a ligand from one minimum to another or the time of the escape of the reaction products from the AS pocket. In 1D case such estimates for noninteracting particles can be done using the Kramers formula. Kramers showed that in 1D relief $U=kx/2$ the escape of a particle from the minimum through the barrier $|x| = x^*$ depends (under the given noise level) on the ratio of the decay coefficient to the eigenfrequency (h/ω). The mean escape time τ is minimal if $h/\omega \sim 1$ and grows exponentially if $h/\omega \rightarrow 0$ and $h/\omega \rightarrow \infty$ [21, 22]. Substantial differences for 2D and 3D cases may result from the possible energy exchange between the vibrations in different directions.

We demonstrated earlier [9, 10, 23] that in the case of the asymmetric potential $U(x,y)$ an effective energy exchange is possible in the system on x and y . In particular, if the ligand (or cluster) of the mass m is «attached to the walls» by four springs of the lengths l and of different rigidities k_i , $i=1, \dots, 4$, forming a cross and if

$$\omega_1 = n\omega_2, \quad \text{where } \omega_1 = [(k_1 + k_3)/m]^{1/2}, \quad \omega_2 = [(k_2 + k_4)/m]^{1/2}, \quad (6)$$

then under $n = 2$ or $1/2$ the Fermi resonance is observed even for small amplitudes $a(a \ll l)$, i.e. the vibrational energy goes periodically with the frequency

$$\Omega \cong 2 \cdot 3^{1/2} (ak_1 - k_3!) / \{l[m(k_2 + k_4)]^{1/2}\} \quad (7)$$

from one orthogonal mode to another and the amplitude increases in this case two times. One can expect even larger amplitude jumps in the case of 3D system.

It goes without saying that such a situation is possible only if the quality factor of the system is very high and the conditions of resonance are met with high accuracy. In the real systems there are noises and damping and the strict fulfillment of the resonance

conditions is hardly possible. On the other hand, the ligands and the clusters acquire energy all the time due to parametric and additive interactions with the large and small clusters of the protein molecule. Such interactions have the color noise nature, i.e. the spectral density of these interactions has maximums at the characteristic frequencies. It was demonstrated [23] that the resonance of Fermi type and, consequently, an effective intermode energy redistribution are possible in the system in the case of harmonic (and quasiharmonic) action at the frequencies coinciding with the resonance ones (ω_1 and ω_2).

The coincidence of the characteristic frequencies of the external action and the resonance frequencies is hardly possible but one should keep in mind that the rigidities k_i may change as a result of, e.g., slow modulation of the length and orientation of the H-bonds by means of which the ligands are bound in the active site of the enzyme. The estimates show that the resonance frequencies of the substrate bound in AS AChE range from 10^{12} to 10^{13} Hz.

To specify Kramers conclusion on the dependence of the escape time « τ » upon h/ω we carried out a computer experiment. The system of equations for the motion of a particle of mass $m = 1$ under the action of the delta-correlated noises ξ_1, ξ_2 in the potential field $U(x,y)$ is

$$\begin{aligned} d^2x/dt^2 &= -\partial U/\partial x - hdx/dt + (D_a h)^{1/2}\xi_1(t), \\ d^2y/dt^2 &= -\partial U/\partial y - hdy/dt + (D_a h)^{1/2}\xi_2(t). \end{aligned} \quad (8)$$

The system was solved for five different cases:

- | | |
|----------------------------------|---|
| 1. $U = x^2 + y^2$, | $\Gamma: U^* = 25$, i.e. $x^2 + y^2 = 25$; |
| 2. $U = x^2 + y^2$, | $\Gamma: U^* = 16$, i.e. $x^2 + y^2 = 16$; |
| 3. $U = x^2 + y^2$, | $\Gamma: U^* = 25$ or $x^* = 4$; |
| 4. $U = x^2 + y^2/4$, | $\Gamma: U^* = 25$ or $x^* = 4$; |
| 5. $U = (x^2 + y^2) f(\alpha)$, | $\Gamma: U^* = 25$ or $x^* = 2 \cdot 2^{1/2}$. |

Here $f(\alpha) = [15/16 - 1/8 \sin \alpha + 1/2 \cos \alpha + 9/16 \cos(2\alpha)]$, $\alpha = \arctg(x/y)$, and « Γ » determines the termination of calculations. Calculations are terminated if at least one of the conditions is met: the potential energy reaches the value U^* or the particle reaches a certain border at the x - y plane. In the cases 3 - 5 this border is determined as a section of a paraboloid by the plane $x = x^*$. In the lowest point of the cross section the potential energy equals 16 and in the case 4 the ratio of the frequencies of the natural oscillations of a particle in the directions x and y is 1:2. However, there is no intermode energy redistribution in this case. In the case 5 potential corresponds to the motion of a particle in the x - y plane under the action of the springs $k_1=2, k_2=1/4, k_3=1, k_4=1/2$ ($f(0)=2; f(\pi/2)=1/4; f(\pi)=1; f(3\pi/2)=1/2$), and only in this case the redistribution of energy is possible.

The topograms of the corresponding relieves $U(x, y)$ are presented in Fig. 4, a. The dependences of t on h/ω for the cases 1 - 5 are presented in Fig. 4, b. It is seen that only for the case 5 the time t decreases substantially due to energy redistribution in comparison with the cases 1-3 under high $Q(\omega/h \geq 10)$.

We performed additional studies of the case 5:

- 5.1 $\Gamma: x^* = 2 \cdot 2^{1/2}$; 5.2 $\Gamma: x^* = -4$; 5.3 $\Gamma: y^* = 8$; 5.4 $\Gamma: y^* = -4 \cdot 2^{1/2}$.

Here in comparison with the case 5 the border of the area has an exit only in a certain direction (in all the other directions the potential barrier is assumed to be infinite). The results of computations are presented in Fig. 5. The triangles in Fig. 5, c correspond to the cases 5.1 and 5.3, the circles correspond to the cases 5.2 and 5.4. It is seen (compare with the case 5) that the escape time depends upon the two-dimensionality of the potential in the case of the existence of the vibrational process (the curves seem to be

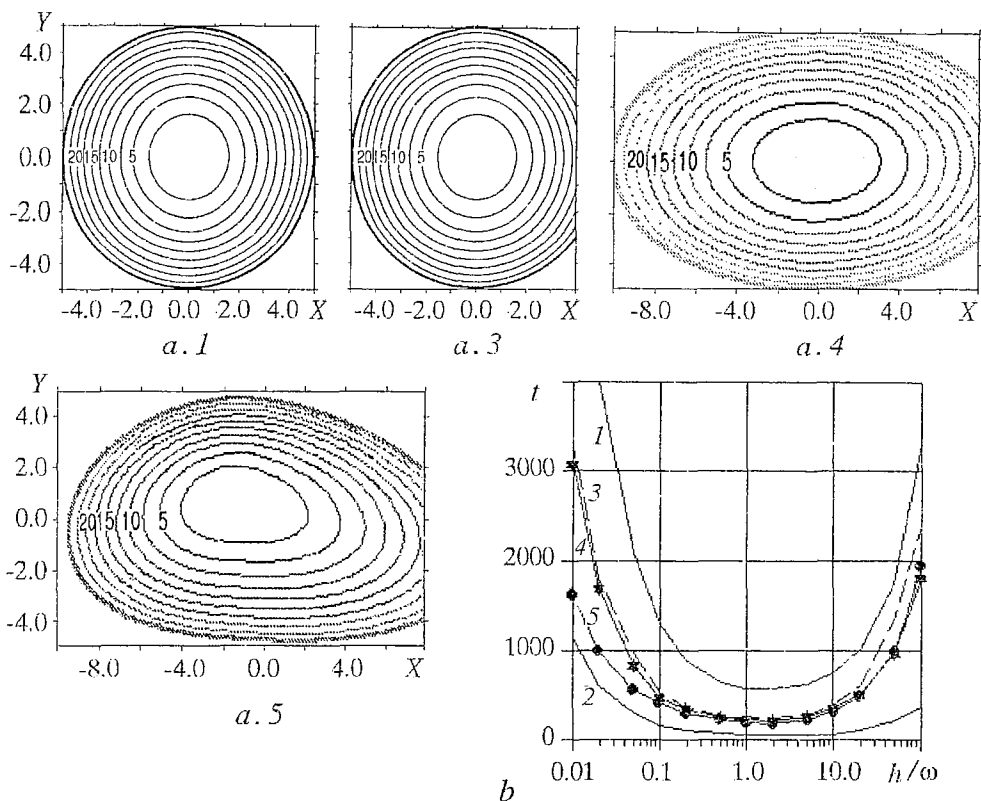


Fig. 4. *a* - topograms of the potential relieves for the cases 1 (*a.1* - the escape is possible in the case $U=U^*=25$); 3 (*a.3* - the escape is possible in the case $U=U^*=25$ or $x>4$); 4 (*a.4* - the escape is possible in the case $U=U^*=25$ or $x>4$); 5 (*a.5* - the escape is possible in the case $U=U^*=25$ or $x>2.2^{1/2}$); *b* - dependence of the mean escape time on the ratio h/ω ($\omega=1$)

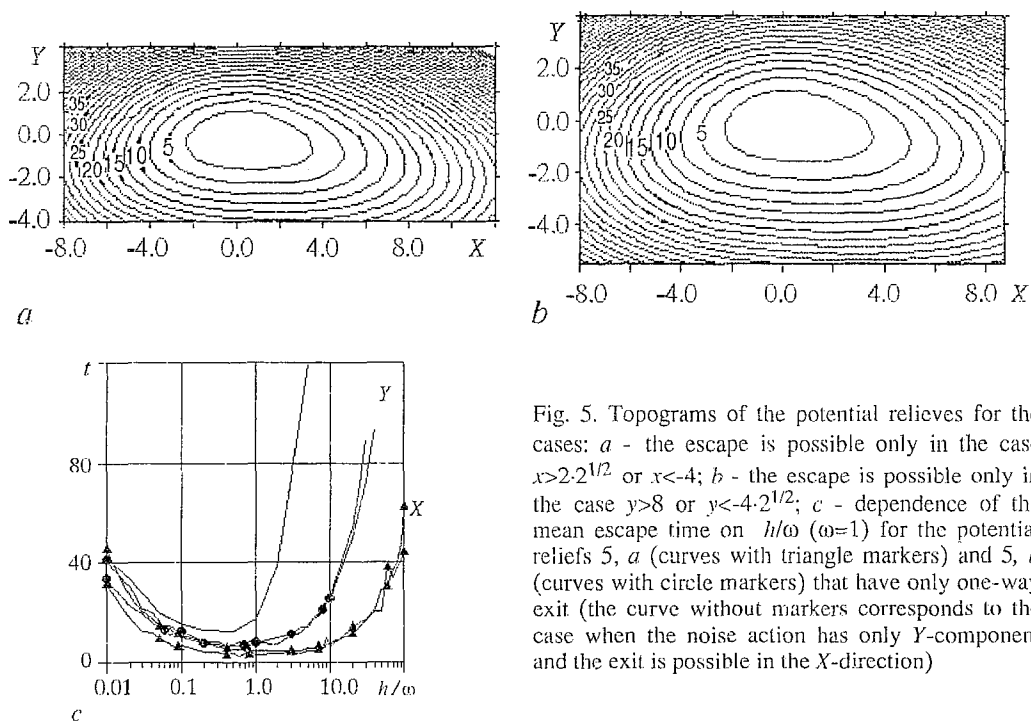


Fig. 5. Topograms of the potential relieves for the cases: *a* - the escape is possible only in the case $x>2.2^{1/2}$ or $x<-4$; *b* - the escape is possible only in the case $y>8$ or $y<-4.2^{1/2}$; *c* - dependence of the mean escape time on h/ω ($\omega=1$) for the potential reliefs 5, *a* (curves with triangle markers) and 5, *b* (curves with circle markers) that have only one-way exit (the curve without markers corresponds to the case when the noise action has only Y-component and the exit is possible in the X-direction)

symmetrical relative to 1 - the frequency of the natural oscillations). Moreover, we considered the *case* (the solid line) for border 5.1 when the disturbing force acts only in the direction of Y axis (under these conditions the particle never crosses the border of the area if there is no intermode energy redistribution). In the considered case the effective energy redistribution (possible for the vibrational process with low damping) allowed the particle to reach the border in nearly the same time as in *cases* 5.1 - 5.4. At the same time if the friction coefficient is rather high (the system is overdamped) there is no vibrational motion in the system and the time of reaching the border increases rapidly. How can we use (at least at the qualitative level) these results for solving the problem of the products escape from the active site? It is likely that they can be used for evaluation of the situation in the active site of ACT [23] where only one ligand resides after breaking of the peptide bond. The situation is much more complex in the case of AChE.

6. The problem of the escape of the reaction products from AS of AChE

The simulation of the escape of the reaction products from the pocket of AS AChE appears to be rather difficult due to the reasons as follows. Firstly, several particles can be found inside the AS pocket: positively charged ACh and Ch and also negatively charged A. It is necessary to take into account their interaction. Secondly, the potential relief inside the AS pocket is of a complex shape and it is rather difficult to determine it accurately. In contrast to the penetration problem its shape is of great importance.

In Fig. 6 we present the graphs of the electrostatic component of the potential $U(z)$ at the axis of AChE molecule for different values of the ionic strength in the AS pocket calculated on the basis of the information from the Protein Data Bank. Besides $U(z)$ one should take into account also the Van-der-Waals forces from the atoms that form the inner surface of the AS pocket.

However, one can be sure that $U(z)$ has a minimum at a distance of several angstroms from the bottom of the pocket. Due to this reason positive ACh and Ch can escape because of random interactions of the particles with each other and with the oscillating atoms of the walls of the AS pocket, whereas the negatively charged A can get into the minimum in the vicinity of the pocket bottom.

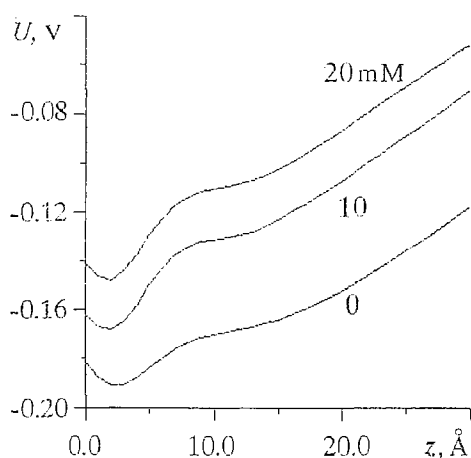


Fig. 6. Electrostatic component of the potential along the axis of the AS AChE pocket for different ionic strengths

Basing on these results we chose a simplified scheme of the charges location in AChE molecule for simulation of the «escape». The first experiments with this model showed that under certain parameters of the 2D electrostatic potential the escape of both positive and negative fragments is influenced by minor oscillations (with certain resonance frequencies) of globules that form the entrance Lennard-Jones potential. The frequencies vary within wide ranges in the case of small variation of the parameters. At the moment of penetration through the barrier the fragment should not meet the attacking dumbbell that can simply bring it back to the active site. Note that the number of attacking dumbbells must be rather large and, hence, the collision at the exit is highly probable. It is highly likely

that the continuous work of the molecular scissors is dependent upon the withdrawal of the negatively charged fragments through the «back door». It is necessary also to estimate the possibility of the escape of A in the case it associates temporarily with H⁺ and becomes neutral. Mean pH in the AS pocket must be known for this reason. Besides that A⁻ may form complex with positive ion Ch⁺. Our estimates show that the bond energy in this case is comparable with the mean kinetic energy of the molecule if we assume that both Ch⁺ and A⁻ have the energy of $3/2kT$.

7. Conclusion

1. We did not touch the methods and the programs using which we solved the equations (4) because they are virtually the same as those used in [7, 11].

2. Our experience shows that in several cases when ten or more particles of complex shape interact with each other it is not necessary to introduce external noises into the model when solving the «escape» problem. The qualitative results remain virtually the same (see also [24, 25]).

3. We only outlined the ways of solving of the «back door» problem. Preliminary results show that the work of AChE is more effective in the case when the escape of the negatively charged products is possible through an additional exit. The solution of this problem seems to be possible only within the frames of the 3D model of AChE.

4. Several important problems related with the proton transfer from one minimum to another arise if one considers the mechanism of breaking of the attacked bond in the substrate A-B localized near the catalytic group of the active site. Such problems were considered for the AS of ACT as classical [12, 10, 7, 11, 13] and quantum ones [4]. They are reduced to the problem of the proton transfer in the potential relief with three minimums. Complementary binding of A-B in the vicinity of the catalytic group seems to influence both the parameters of the barriers between the potential wells and their depths. The barrier for the proton transfer in the H-bond O-H...N is rather high (more than $40 kT$) and, hence, the transfer is likely to take place via the third minimum [26]. After the proton transfer the oxygen atom acquires negative charge. The successful attack of the bond by the negatively charged oxygen is possible in the case of their getting closer to each other which is possible due to intense oscillations of the substrate and oxygen atom relative to each other (both for ACT and AChE). An effective energy redistribution under nonlinear interaction of the vibrational modes may play an important role. Note that the problem of proton excitation in the H-bond is discussed also for different situations [27].

5. We developed a series of computer codes that make it possible to take into account, firstly, the sum electrostatic action of all the dumbbells on each dumbbell and, secondly, the noise action on and the corresponding friction coefficients for the dumbbell resulting from the impacts of the water molecules. Thus, it will be possible to relate our results with the statistical theory of dipole-dipole interactions based on the solution of the Langevine equations and the corresponding Kolmogorov - Fischer - Planck equations.

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*Department of Physics, Moscow State University
Institute of Mechanics, Moscow State University*

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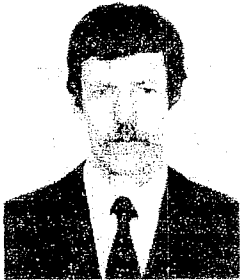
НЕКОТОРЫЕ ЗАДАЧИ КЛАСТЕРНОЙ ДИНАМИКИ: МОДЕЛЬ МОЛЕКУЛЯРНЫХ НОЖНИЦ

Ю.И. Романовский, А.В. Нетребко

Методами молекулярной динамики ставится и решается ряд задач броуновского движения частиц - гантелей А-В, которые могут распадаться на части А и В. В качестве примера рассматривается задача о «разрезании» положительного заряженного ацетилхолина А-В на две части А и В в активном центре гидролитического фермента ацетилхолинэстеразы. Модели строятся на основе данных из Protein Data Bank. Определены условия диффузионного ограничения скорости работы АСhЕ по «входу» А-В в карман активного центра фермента и выхода из него А и В.



Romanovsky Yury Mikhailovich, born in 1929, graduated from Physics Department of Moscow State University in 1952. Doctor of Physics and Mathematics. Full professor of Physics Department of MSU, head of laboratory of laser and mathematical biophysics. The author of 6 monographs, devoted to the dynamics of nonlinear systems and mathematical biophysics.
E-mail: romanov@lbp.ilc.msu.su



Netrebko Alexey Vasilievich, born in 1955, graduated from Mechanics and Mathematics Department of Moscow State University in 1977. Ph.D. in Physics and Mathematics. Leader researcher of Institute of Mechanics of MSU. Scientific interests: dynamic theory of plasticity, mathematical simulations in mechanics of solid bodies, numerical methods. The author of more than 30 scientific works.
E-mail: netrebko@inmech.msu.su