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ORIGINAL ARTICLE

Some possible peculiarities of a ligand molecule binding inside of a protein macromolecule

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Abstract: **This article proposes a model for a ligand molecule binding inside of a protein macromolecule. The model considers peculiarities of the ligand access to a buried binding center of the protein. The number of the ligand molecule possible trajectories inside of an access channel leading to the binding center is limited by the channel. Therefore average velocity of the ligand molecule translocation inside of an access channel increase with the decreasing of its inner diameter. Thus during overcoming of the activation barrier between neighboring balanced positions inside of the channel, the ligand molecule should dissipate certain amount of its energy. In other words it should also overcome a drag barrier of activation, which is thus a component of the activation barrier. Our calculation showed that the drag barrier of activation is proportional to the temperature and to the squared ratio of the ligand diameter and the distance-averaged channel diameter. Due to possible gradient of the channel inner diameter the drag barriers of activation for the forward and backward ligand translocations inside of the channel may not equal one another. It follows from this that the binding constant depends also on the channel shape.**

Keywords: **binding center, binding constant, ligand, protein, protein access channel**

I. Introduction

All vital proteins are able to selectively bind different molecules called ligands. This ability enables proteins to fulfill different functions [1]. For example, hemoglobin located in erythrocytes binds and carries O_2 from lungs to all tissues by the blood stream and in the same time it binds and carries CO_2 in the opposite direction [2,3]. Myoglobin the other important hemoprotein located in muscle cells stores O_2 and facilitates its diffusion [4, 5]. Protein transmembrane channels and pumps transport different ions through biological membranes correspondingly passively and actively. In this way they play key roles in different vital cell physiological processes such as electric transmembrane potential, neural impulse et cetera [1,6]. Due to the ligand binding the proteins catalyze almost all known biochemical reactions [1, 7]. Protein receptors accepting hormone signal transmit it to the protein switches, which change physiological activity of a whole cell [1, 8]. The binding of proteins actin and myosin causes muscle contraction, active cell movement and organelles traffic. Protein antibodies are able to specifically bind different antigens on surfaces of pathogenic viruses and bacteria for preventing their growth and development in an organism [1]. Thus clear understanding of ligands binding processes with proteins is necessary for the advancement of medicine.

If specific binding sites for certain ligands are located on the protein surface, they are accessible for the ligand molecules of all possible sizes [1]. When the binding sites are buried inside of proteins internal cavities, they are accessible mostly for ligand molecules considerably smaller than the protein ones. Thus in the latter case small ligands should get to the protein binding center via networks of crevices and cavities created by the foldings of the polypeptide chains. Because such

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crevices and cavities are constructed by more or less rigor polypeptide backbone the ligands pathways to and out of protein intramolecular binding sites are relatively stable.

Mentioned crevices or cavities connected in a chain are called ligand channel [9]. The cavities connected by the crevices into a net are called ligand migration network [10] or channels network [11]. In the case, when a ligand binded by a protein simultaneously is a substrate of certain biochemical reaction catalyzed by this protein the ligand pathways are called substrate channels [9] or substrate tunnels [12]. From among 4,306 enzymes analyzed 86.8% of them contained channels longer than 5 Å, while over 64% had two or more channels longer than 15 Å. The channels studied average length exceeded 25 Å. In other words majority of the channels are considerably longer than sizes of correspondent ligands [7].

It was shown that hemoglobin and myoglobin have a system of ligand migration networks allowing O_2, CO_2 and other ligands freely access to the buried binding sites [10]. All transmembrane protein channels and pumps have corresponding ligand channels for the transmembrane transport [1,6,13–15]. Analogically one can say the same about receptor proteins. For example G-protein-coupled receptor has a ligand tunnel of considerable length within 20 - 35 Å [8]. It was stated that the channels play a key role in the substrate affinity of enzymes [7,12]. Therefore, their structure and dynamics have been extensively studied lately [7, 10–12].

It is clear that inside of an access channel the number of ligand molecule possible trajectories is smaller in comparison with the ligand in an unbound medium, see Fig. 1.

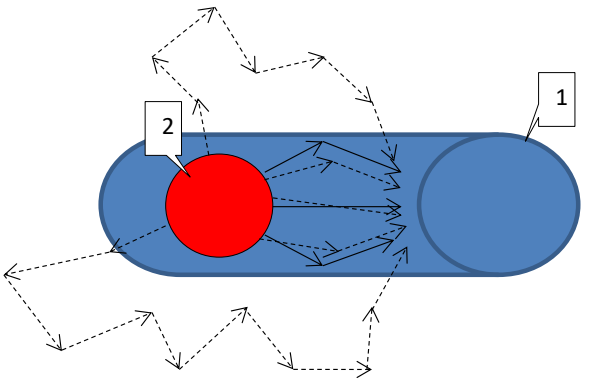

Fig. 1: Schematic representation of a ligand molecule translocation. Solid arrows mark some possible trajectories of the ligand inside of a protein access channel. Dashed arrows mark some possible trajectories in the case, when the ligand moves in an unbound medium. 1 is the protein access channel, 2 is the ligand molecule.

Translocation of the particles via limited number of possible trajectories has an important peculiarity: the value of average velocity of the translocation $\overline{\dot{x}}_{av}$ is not negligible. $\overline{\dot{x}}_{av}$ is proportional to the relation of the ligand molecule volume V_m and the volume, which is free accessible for the particles V_{ac} [16]:

$$\overline{\dot{x}}_{av} \neq 0,\ \overline{\dot{x}}_{av} \propto \frac{V_m}{V_{ac}}. \qquad (1)$$

Thus due to the friction and to (1) a ligand equilibrial translocation inside of an access channel is always connected with energy dissipations. According to (1) and that the drag force depends on the velocity, it is obvious that the value of such energy dissipations depends on the ligand and channel shapes and sizes.

For the movement of a small molecule through any condensed matter (excluding only superfluids) including native proteins and carbon nanotubes, such a small molecule should periodically "jump up" the activation barriers [10,17]. The activation barrier usually is characterized by the Gibbs free energy of activation, $\Delta G^{\ddagger}$, which has to be overcome by a molecule to take a new balanced position in neighbor free volume [17]. The molecule overcomes the activation barrier only at a part of the distance between both balanced positions. Further without the fluctuation force intervention the molecule moves only within the new balanced position. Thus taking into account mentioned in the previous paragraph we can state that the activation barrier for the ligand diffusion inside of a protein access channel is higher than $\Delta G^{\ddagger}$ (which does not evaluate any friction processes) due to the dissipation. The dissipation amount necessary for one mole of the ligand molecules to overcome $\Delta G^{\ddagger}$ barrier let us call by a drag barrier of activation.

As it was shown the protein channels often have inner diameter gradient [1, 10, 14]. Due to it we can assume that the drag barriers of activation for the forward and backward ligand movement in such channels may not equal one another, see Fig. 2.

Thus it may generate spontaneous directional motion of ligand molecules inside of a protein channel with the inner diameter gradient without resorting to any active driving source, such as temperature, electrical or chemical gradients. Analogical spontaneous directional motion of water molecules was shown in single-walled carbon nanotubes with a stiffness gradient [18]. It is clear that such a phenomenon impacts on the protein

ligand binding capability.

As it is known the ligand binding capability is characterized by the binding constant. Usually it is calculated under the conditions of 1) equilibrium, 2) zero average velocities of the ligand equilibrial translocation to and out of the binding center. In an unbound medium the latter condition emerges from the first one. Thus according to the Gibbs distribution [19] the binding constant is calculated in such a way [20]:

$$K_{bin} \equiv \frac{[L \cdot P]}{[L] \cdot [P]} = \exp\left(- \frac{\Delta G}{R \cdot T} \right), \qquad \Delta G \equiv G_{L \cdot P} - G_{L+P}, \tag{2a}$$

where $[L]$, $[P]$ and $[L \cdot P]$ are equilibrium concentrations of the ligand, protein and ligand-protein complex respectively, T is the temperature, R is the molar gas constant, $G_{L \cdot P}$ is the Gibbs free energy of the ligand-protein complex, G_{L+P} is the Gibbs free energy of the solution of separated ligand and protein molecules. The Gibbs free energies difference ΔG for a ligand binding with a protein in formula (2a) can also be written in a form:

$$\Delta G = - \sum_{n=1}^{N-1} \Delta(\Delta G_n^{\ddagger}), \qquad \Delta(\Delta G_n^{\ddagger}) \equiv \Delta G_{fn}^{\ddagger} - \Delta G_{bn}^{\ddagger}, \tag{2b}$$

where $\Delta G_{fn}^{\ddagger}, \Delta G_{bn}^{\ddagger}$ are the Gibbs free energies of activation of the ligand moving from n-th balanced position to $n+1$-th one (in the direction from the binding center, where let $n = 1$) and backward correspondingly, N is the number of the balanced positions for the ligand molecule inside of the channel. As a function of thermodynamic state, the Gibbs free energy does not take into account the friction and hence the drag barrier [19]. But the drag barriers should also be a part of the diffusion activation energy.

Thus analogically with (2b) we can supplement formula (2a) in such a form:

$$K_{bin} = \exp\left(\frac{-\Delta G + \sum_{n=1}^{N-1} \Delta(\Delta \Gamma_n^{\ddagger})}{R \cdot T} \right), \qquad \Delta(\Delta \Gamma_n^{\ddagger}) \equiv \Delta \Gamma_{fn}^{\ddagger} - \Delta \Gamma_{bn}^{\ddagger}, \tag{2c}$$

where $\Delta \Gamma_{fn}^{\ddagger}, \Delta \Gamma_{bn}^{\ddagger}$ are the drag barriers of activation for the ligand molecule moving from n-balanced position to $n+1$ one (in the direction from the binding center, where let $n = 1$) and backward correspondingly (Fig. 2). The condition of zero average velocities of the ligand translocation to and out of the binding center does not have to be fulfilled for validity of formula (2c). Therefore formula (2c) is more suitable for the binding

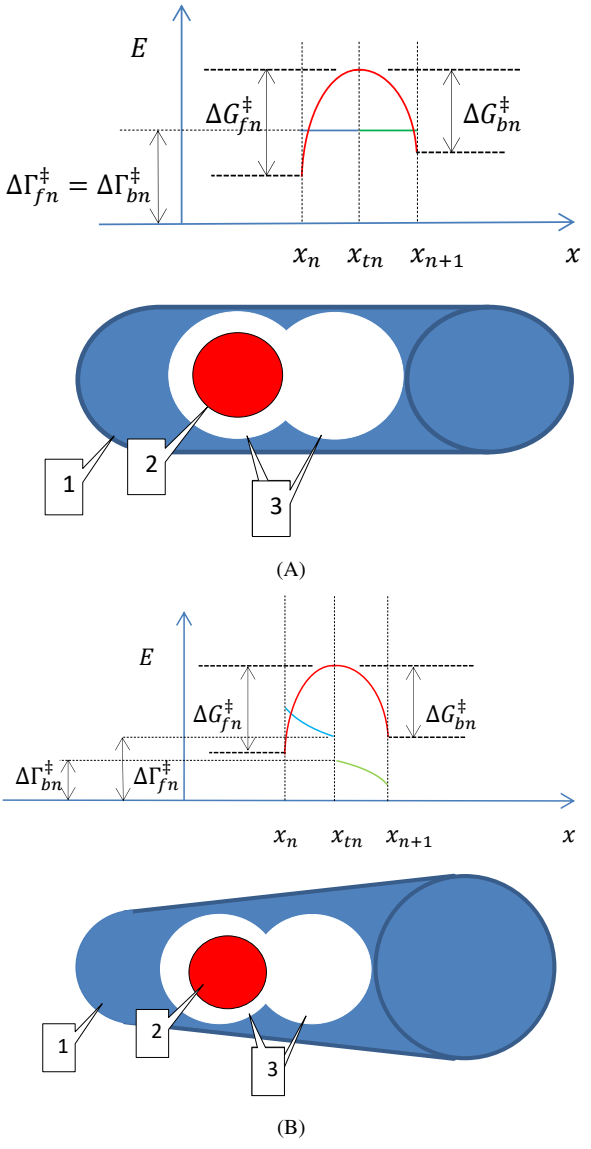

Fig. 2: Schematic representation of an energetic profile of a system during a ligand molecule activation jumping from n-th balanced position to $n+1$-th one and backward inside of a protein access channel. A – energetic profile in a cylindrical channel. B – energetic profile in an expanding channel. Red lines mark the Gibbs free energy of the system. Blue lines mark average dissipation energy amount of one mole of the ligand molecules (the drag barrier) moving from n-th balanced position (with coordinate x_n) to the threshold point x_{tn}. Green lines mark the drag barrier for the ligand moving from $n+1$-th balanced position (with coordinate x_{n+1}) to the threshold point x_{tn}. 1 is the protein access channel, 2 is the ligand molecule, 3 is the free volume, $\Delta G_{fn}^{\ddagger}$ and $\Delta G_{bn}^{\ddagger}$ are the Gibbs free energies of activation for the forward and backward ligand movement correspondingly, $\Delta \Gamma_{fn}^{\ddagger}$ and $\Delta \Gamma_{bn}^{\ddagger}$ are the drag barriers of activation for the forward and backward ligand movement respectively.

constant calculations for a protein with a buried binding site. As it was already mentioned the drag barriers of activation depend on the channel shape.

Thus as the ligand channels play an important role in the protein functioning [7,10], we can easily assume that their geometries can also considerably impact on the binding constant as formula (2c) shows. This would allow various natural effectors [4, 21] to more effectively regulate protein functions changing also access channels shape and size. Let us try to clarify this question considering in the details: 1) a ligand movement inside of an access channel, 2) a ligand overcoming of the diffusional activation barrier inside of the channel.

II. Model description

Let us consider the movement of a spherical ligand molecule inside of a protein access channel. Because the curvature of most of such channels is not so high [9–12] let us consider the model channel as an axial symmetric one. The channel's endpoints are the binding center and the adsorption center on the protein surface (Fig. 3).

The stochastic motion of a ligand molecule along the channel symmetry axis x can be described by the Langevin equation [22]:

$$m\ddot{x} + h\dot{x} + F(x) = A(t), \tag{3}$$

where m and h are the ligand mass and friction coefficient correspondingly, $\dot{x}$ and $\ddot{x}$ are the ligand molecule velocity and acceleration respectively, $F(x)$ and $A(t)$ are the potential and fluctuation forces correspondingly. Assume that the channel medium can be modelled as a Newtonian fluid so that h is independent from time, coordinate and velocity. The friction coefficient h depends on the ligand diameter as well on protein intramolecular organization and dynamics.

Now let us integrate equation (3) with respect to coordinate and then average it with respect to the ensemble of a multitude of identical systems analogically to the proposed [16, 23]. In the state of equilibrium according to the law of equipartition, the average kinetic energy of the particle

$$E_k = \frac{m\overline{\dot{x}}^2}{2} = \frac{1}{2}k_bT,$$

is independent from coordinate, $\overline{\dot{x}}$ is the average velocity of the ligand translocation. k_b is the Bolzman constant, T is thermodynamic temperature.

Taking all that into account, we obtain:

$$I(x_1, x_2) = P(x_2) - P(x_1) - \Gamma(x_1, x_2), \tag{4}$$

where

$$I(x_1, x_2) \equiv \int_{x_1}^{x_2} \overline{A}(x)dx$$

is the average work done by the fluctuation force for translocation of the ligand molecule from point x_1 to point x_2, $\overline{A}(x)$ is the mean fluctuation force, also

$$\Gamma(x_1, x_2) \equiv -h \cdot \int_{x_1}^{x_2} \overline{\dot{x}}(x)dx$$

is the average work done by the drag force during the translocation of the ligand molecule from point x_1 to point x_2, $P(x_1)$ and $P(x_2)$ are the ligand potential energies in points x_1 and x_2 respectively.

In an unbound fluid due to multiplicity of possible translocation trajectories of a ligand we have $\overline{\dot{x}} = 0$ for all $x \in [x_1, x_2]$. According to (4), the average work of the fluctuation force, which should be made for the ligand translocation between points x_1 and x_2, equals the difference of the ligand potential energies in both points.

The channel limits the number of the possible trajectories (Fig. 1). According to [16], ligand translocation velocity $\overline{\dot{x}}_{av}$, averaged with respect to the ensemble of identical systems and coordinates, in the internal volume of the channel, which is the accessible volume for the ligand molecule, can be calculated as:

$$\overline{\dot{x}}_{av} \equiv \frac{1}{x_2 - x_1}\int_{x_1}^{x_2} \overline{\dot{x}}(x)dx \sim \frac{x_2 - x_1}{|x_2 - x_1|}\frac{V_m}{V_{ac}} \cdot \overline{\nu},$$
$$\overline{\nu} \sim \frac{D}{d}, \quad D = \frac{k_b \cdot T}{h}, \tag{5}$$

where $\overline{\nu}$ is the mean velocity of the ligand on the distance close to its dimensions, d is the ligand diameter, D is the coefficient of diffusion of the ligand.

Let us evaluate the quantity $\Gamma(x_1, x_2)$ in formula (4) using formula (5). Thus we obtain:

$$\Gamma(x_1, x_2) = \overline{\dot{x}}_{av} \cdot (x_1 - x_2) \cdot h \sim -\frac{V_m \cdot |x_2 - x_1|}{V_{ac} \cdot d} \cdot k_b \cdot T. \tag{6}$$

Formula (6) demonstrates that average work done by the drag force during an equilibrial translocation of the ligand inside of an access channel is independent from peculiarities of protein intramolecular dynamics (in other words it is independent from h). Taking into account formula (4), the same can be said about average work done by the fluctuation force I, which is always connected with Γ.

For a spherical ligand we have:

$$V_m = \frac{1}{6} \cdot \pi \cdot d^3. \tag{7a}$$

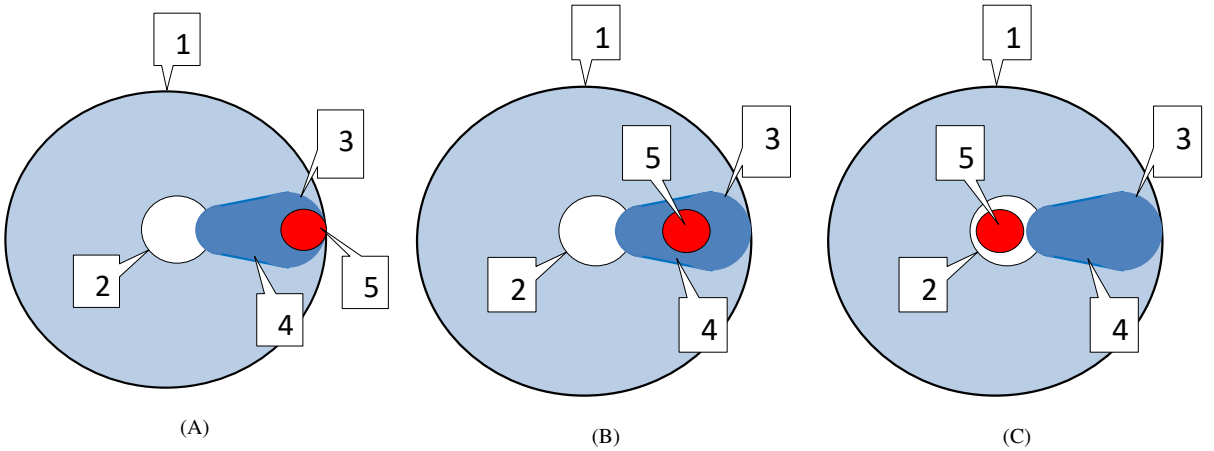

Fig. 3: Schematic representation of binding of a ligand molecule with a protein macromolecule. A, B, C are different stages of the binding. 1 is the protein globular macromolecule, 2 is the binding center, 3 is the adsorption center, 4 is the protein access channel, 5 is the ligand molecule.

For an axially symmetric channel we have:

$$V_{ac} = \frac{1}{4}\pi \int_{x_1}^{x_2} d_c^2(x)dx, \tag{7b}$$

where $d_c(x)$ is the channel inner diameter in the point x. According to (7a) and (7b) we can rewrite formula (6) as:

$$\Gamma(x_1, x_2) \sim -\frac{2 \cdot d^2}{3 \cdot \widetilde{d_c^2}(x_1, x_2)} \cdot k_b \cdot T, \tag{8a}$$

$$\widetilde{d_c^2}(x_1, x_2) = \frac{1}{x_2 - x_1} \int_{x_1}^{x_2} d_c^2(x)dx, \tag{8b}$$

where $\widetilde{d_c^2}(x_1, x_2)$ is the mean square diameter of the channel.

Thus, according to (8a) and (8b) for a ligand equilibrial translocation inside of the channel, on a distance not less than the ligand diameter, the drag force should always do work. The value of such work Γ is independent from the translocation distance and direction. It is proportional to the temperature, and to the squared ratio of the ligand diameter, and the distance-averaged inner channel diameter.

According to the law of the symmetry of movement under time reversal transformation, for the forward and backward average velocities of ligand equilibrial translocations, in each point inside of the channel ($\overline{\dot{x}}_f(x)$ and $\overline{\dot{x}}_b(x)$ respectively) we always have:

$$\overline{\dot{x}}_f(x) = -\overline{\dot{x}}_b(x). \tag{9}$$

Thus, taking into account (5), (7a), (7b) we can approximate the velocities modules as unambiguous coordinate functions:

$$|\overline{\dot{x}}_f(x)| = |\overline{\dot{x}}_b(x)| \approx \frac{1}{|x_2 - x_1|} \cdot \frac{2 \cdot d^2}{3 \cdot d_c^2(x)} \cdot \frac{k_b \cdot T}{h}. \tag{10}$$

According to (5) and (10), approximation (8a) may be improved, where we get instead of (8b):

$$\widetilde{d_c^2} = (x_2 - x_1) \cdot \left(\int_{x_1}^{x_2} d_c^{-2}(x)dx \right)^{-1}. \tag{11}$$

A. The role of the drag barrier of activation in a ligand diffusion inside of a protein access channel. Appearance of a drag pseudopotential inside of the channel.

All diffusion processes in usual condensed matter are always characterized by the activation energy. The value of the activation energy usually is characterized by the Gibbs free energy of activation $\Delta G^{\ddagger}$, required for one mole of molecules to overcome an energetic barrier between two neighbor balanced positions [17], see Fig. 2.

Moving through the whole channel the ligand molecule should gradually go via N balanced positions. Locations of such balanced positions may clearly correlate with the locations of certain amino acid residues of the channel, as it was shown by Diamantis et al. [10] for hemoglobin, and by Pfeffermann et al. [17] for aquaporin. But such a correlation is not compulsory, as it was shown by Nonner and Eisenberg [14] for L-Type of Ca^{2+}-channels. For the ligand molecule to overcome the barrier between n-th and $(n+1)$-th balanced positions, it is enough to move only a part of

the distance between the positions (to cross over the threshold point, which we mark by x_{tn}, see Fig. 2).

The ligand molecule, which has already overcome the threshold point, without an intervention of the fluctuation force moves only within the new position. The same can be said about the backward movement. Thus, the ability of the ligand molecule to translocate between the balanced positions is determined by its ability to cross over the threshold points x_{tn}. The Gibbs free energy is a state function independent from friction processes [19]. Therefore, according to the previous section in the article, inside of the channel the value of the ligand diffusion activation energy E_{Afn} should be higher than $\Delta G^{\ddagger}_{fn}$:

$$E_{Afn} = \Delta G^{\ddagger}_{fn} + \Delta\Gamma^{\ddagger}_{fn}, \quad \Delta\Gamma^{\ddagger}_{fn} > 0. \tag{12}$$

Let us call $\Delta\Gamma^{\ddagger}_{fn}$ a drag barrier of activation. It equals the modulus of the work, which should be done by the drag force for translocation of one mole of the ligand molecules from n-th balanced position with coordinate x_n to a threshold point x_{tn}:

$$\Delta\Gamma^{\ddagger}_{fn} \equiv N_A \cdot |\Gamma(x_n, x_{tn})|,$$

where N_A is the Avogadro constant.

According to formulas (4) and (8a) $\Delta\Gamma^{\ddagger}_{fn}$ has a fluctuation nature. The ligand diffusion activation energy E_{Afn}, which is the sum of $\Delta G^{\ddagger}_{fn}$ and $\Delta\Gamma^{\ddagger}_{fn}$ (12), equals certain value of energy, which should be given to one mole of the ligand molecules from heat bath for overcoming the activation barrier between n-th and $(n+1)$-th balanced positions. Note that according to the written after formula (2c), the first balanced position is the nearest to the binding center.

Under the gradient of the channel inner diameter, according to (8a), (11), the drag barriers of activation for the ligand forward and backward translocations between n-th and $(n+1)$-th balanced positions may not equal one another (Fig. 2):

$$\Delta\Gamma^{\ddagger}_{fn} \neq \Delta\Gamma^{\ddagger}_{bn}, \tag{13a}$$

where $\Delta\Gamma^{\ddagger}_{bn}$ is the drag barrier of activation for the backward equilibrial translocation of one mole of the ligand molecules:

$$\Delta\Gamma^{\ddagger}_{bn} \equiv N_A \cdot |\Gamma(x_{n+1}, x_{tn})|.$$

Although the works of the drag force during the forward and backward equilibrial translocations always equal one another: $\Gamma(x_n, x_{n+1}) = \Gamma(x_{n+1}, x_n)$, but these quantities do not determine all possibilities of the forward and backward translocations. It is $\Delta G^{\ddagger}_{fn}$ with $\Delta\Gamma^{\ddagger}_{fn}$ and $\Delta G^{\ddagger}_{bn}$ with $\Delta\Gamma^{\ddagger}_{bn}$ correspondingly, which determine such possibilities.

As it was already mentioned, $\Delta\Gamma^{\ddagger}_{fn}$ and $\Delta\Gamma^{\ddagger}_{bn}$ are determined by formulas (8a) and (11), where integrating margins are x_n, x_{tn} and x_{n+1}, x_{tn} respectively:

$$\begin{aligned} \Delta\Gamma^{\ddagger}_{fn} &\sim \frac{2 \cdot d^2 \cdot \int_{x_n}^{x_{tn}} d_c^{-2}(x)dx}{3 \cdot (x_{tn} - x_n)} \cdot R \cdot T, \\ \Delta\Gamma^{\ddagger}_{bn} &\sim \frac{2 \cdot d^2 \cdot \int_{x_{n+1}}^{x_{tn}} d_c^{-2}(x)dx}{3 \cdot (x_{tn} - x_{n+1})} \cdot R \cdot T. \end{aligned} \tag{13b}$$

Let the channel inner diameter depend linearly on coordinate x on the interval between both balanced positions x_n and x_{n+1}:

$$d_c(x) = \delta + a_n x, \quad a_n \neq 0, \quad d_c(x) > d. \tag{14a}$$

In the case when the channel diameter increases with the coordinate increasing, we have:

$$a_n > 0, \quad \delta > d - a_n \cdot \inf\{x_n, x_{n+1}\}. \tag{14b}$$

If it decreases with the coordinate increasing, then we get:

$$a_n < 0, \quad \delta > d - a_n \cdot \sup\{x_n, x_{n+1}\}. \tag{14c}$$

Let us write the difference of the drag barriers of activation for the ligand forward and backward translocations between n-th and $(n+1)$-th balanced positions in such a form:

$$\Delta(\Delta\Gamma^{\ddagger}_n) \equiv \Delta\Gamma^{\ddagger}_{fn} - \Delta\Gamma^{\ddagger}_{bn}. \tag{15}$$

According to formulas (13b), (14a) and (15) we have $\Delta(\Delta\Gamma^{\ddagger}_n) \neq 0$, which confirms formula (13a). It allows us to input new quantity – the drag pseudopotential of the ligand in n-th balanced position inside of the channel, $\Gamma_p(n)$. The difference of the drag pseudopotentials of $(n+1)$-th and n-th balanced positions equals $\Delta(\Delta\Gamma^{\ddagger}_n)$:

$$\Delta(\Delta\Gamma^{\ddagger}_n) = \Gamma_p(n+1) - \Gamma_p(n). \tag{16a}$$

Now let us find $\Delta(\Delta\Gamma^{\ddagger}_n)$. Define:

$$b_n \equiv \frac{x_{tn} - x_n}{x_{n+1} - x_n}, \quad c_n \equiv \frac{x_{n+1} - x_n}{d_c(x_n)}, \quad e_n \equiv \frac{d}{d_c(x_n)}. \tag{16b}$$

According to (13b), (14a), and (16b) we have:

$$\Delta\Gamma^{\ddagger}_{fn} \approx \frac{2}{3} a_n^{-1} \frac{e_n^2}{b_n c_n} \Big(1 - (1 + a_n b_n c_n)^{-1}\Big) \cdot R \cdot T, \tag{17a}$$

$$\begin{aligned} \Delta\Gamma^{\ddagger}_{bn} \approx \frac{2}{3} a_n^{-1} \frac{e_n^2}{c_n(1 - b_n)} \Big((1 + a_n b_n c_n)^{-1} \\ - (1 + a_n c_n)^{-1}\Big) \cdot R \cdot T. \end{aligned} \tag{17b}$$

According to (15), (17a) and (17b) we have:

$$\Delta(\Delta\Gamma_n^{\ddagger}) \approx \frac{2e_n^2}{3a_nc_n} \cdot \left(\frac{(1+a_nc_n)^{-1}}{1-b_n} + b_n^{-1} - \frac{(1-b_n)^{-1}+b_n^{-1}}{1+a_nb_nc_n} \right) \cdot R \cdot T. \tag{18}$$

Let us clarify the dependence of the function $\Delta(\Delta\Gamma_n^{\ddagger})(a_n, b_n, c_n, e_n)$ in formula (18) on a_n, b_n, c_n, e_n.

According to (14a) a_n is a coefficient of linear changes of the channel diameter. For protein access channels we can consider that $0 < |a_n| < 1/3$ for all balanced positions [10, 14, 24].

According to (16b) b_n is the relation of the threshold point location to the distance between n-th and $(n+1)$-th balanced positions, it means that we have $0 < b_n < 1$. Real protein structure imposes restrictions on b_n interval [10]. Thus we can assume that: $1/3 < b_n < 3/4$.

According to (16b) c_n is the relation of the distance between n-th and $(n+1)$-th balanced positions to the channel diameter in the n-th balanced position. Taking into account real protein structure we can assume that $1/2 \le |c_n| \le 2$ [10].

According to (16b) e_n is the relation of the ligand molecule diameter to the channel diameter in the n-th balanced position. For the specific ligands and correspondent access channels we can consider that $1/2 \le e_n < 1$ [10, 14, 17]. From inequality $d_c(x) > d$ in formula (14a) using the determined from (16b) it follows that $a_nc_n - e_n > -1$.

On mentioned intervals of a_n, b_n, c_n, e_n the function $\Delta(\Delta\Gamma_n^{\ddagger})(a_n, b_n, c_n, e_n)$ increases with the increasing of a_n, b_n, c_n, e_n. The sign of $\Delta(\Delta\Gamma_n^{\ddagger})$ is the same as the sign of a_nc_n. It means that if the channel between the n-th and $(n+1)$-th balanced positions expands during increasing of n, then $\Delta(\Delta\Gamma_n^{\ddagger}) > 0$, and when it narrows, then $\Delta(\Delta\Gamma_n^{\ddagger}) < 0$. It is also natural to assume that $b_n = 1/2$ for all balanced positions. Thus formula (18) transforms to:

$$\Delta(\Delta\Gamma_n^{\ddagger}) \approx \frac{4e_n^2}{3a_nc_n} \cdot \left(\frac{1}{1+a_nc_n} + 1 - \frac{2}{1+\frac{1}{2}a_nc_n} \right) \cdot R \cdot T. \tag{19a}$$

Let the channel inner diameter changes in sync with coordinate, so we have $a_n = a = const$ for all balanced positions. Now let us mark the balanced positions again, so that at the first one the channel has the smallest diameter. In this case the first balanced position does not necessarily correspond with the binding center. Thus, let us mark the difference of the drag barriers of activation calculated in the proposed way as $\Delta(\Delta\Gamma_n^{\ddagger *})$.

According with the written before formula (19a) we always have $\Delta(\Delta\Gamma_n^{\ddagger *}) > 0$. For such numbering of the balanced positions it is natural to assume that $d_c(x_1^*) \sim d$, $|c_1^*| \sim 2$, $e_1^* \sim 1$. Let also $(x_{n+1} - x_n) = const$ for all balanced positions. Therefore according to (14a) and (16b) we get:

$$|c_n^*| \sim \frac{2}{1+2|a|(n-1)},\ e_n^* \sim \frac{1}{1+2|a|(n-1)} \tag{19b}$$

and according to (19b) formula (19a) transforms to:

$$\Delta(\Delta\Gamma_n^{\ddagger *}) \approx \frac{2}{3|a|(1+2(n-1)|a|)} \cdot \left(\frac{1}{1+\frac{2|a|}{1+2(n-1)|a|}} + 1 - \frac{2}{1+\frac{|a|}{1+2(n-1)|a|}} \right) \cdot R \cdot T. \tag{19c}$$

Formula (19c) shows that $\Delta(\Delta\Gamma_n^{\ddagger *})$ always decreases with the increasing of n beginning from 1. Thus, taking into account formula (19c) we get:

$$\Delta(\Delta\Gamma_1^{\ddagger *}) \approx \frac{2}{3|a|} \cdot \left(\frac{1}{1+2|a|} + 1 - \frac{2}{1+|a|} \right) \cdot R \cdot T, \tag{20a}$$

$$\Delta(\Delta\Gamma_2^{\ddagger *}) \approx \frac{2}{3|a|(1+2|a|)} \cdot \left(\frac{1}{1+\frac{2|a|}{1+2|a|}} + 1 - \frac{2}{1+\frac{|a|}{1+2|a|}} \right) \cdot R \cdot T, \tag{20b}$$

$$\ldots \tag{20c–20f}$$

$$\Delta(\Delta\Gamma_7^{\ddagger *}) \approx \frac{2}{3|a|(1+12|a|)} \cdot \left(\frac{1}{1+\frac{2|a|}{1+12|a|}} + 1 - \frac{2}{1+\frac{|a|}{1+12|a|}} \right) \cdot R \cdot T, \tag{20g}$$

where we have omitted $n = 3 \ldots 6$ for brevity. As it was shown by Samson and Deutch [24] and Diamantis, et al. [10], real access channels have a slight inner diameter gradient, so we can consider that $|a| \approx 0.1$. Thus according to (20a) – (20g) we have:

$$\begin{aligned} &\frac{\Delta(\Delta\Gamma_1^{\ddagger *})}{R \cdot T} \approx 0.100, \quad \frac{\Delta(\Delta\Gamma_2^{\ddagger *})}{R \cdot T} \approx 0.060, \\ &\frac{\Delta(\Delta\Gamma_3^{\ddagger *})}{R \cdot T} \approx 0.037, \quad \frac{\Delta(\Delta\Gamma_4^{\ddagger *})}{R \cdot T} \approx 0.025, \\ &\frac{\Delta(\Delta\Gamma_5^{\ddagger *})}{R \cdot T} \approx 0.016, \quad \frac{\Delta(\Delta\Gamma_6^{\ddagger *})}{R \cdot T} \approx 0.010, \\ &\frac{\Delta(\Delta\Gamma_7^{\ddagger *})}{R \cdot T} \approx 0.009. \end{aligned} \tag{21}$$

According to formula (16a) the difference of the drag pseudopotentials on the ends of the whole channel $\Delta\Gamma_p$ equals the sum of the differences of the drag barriers of activation for all balanced positions:

$$\Delta\Gamma_p = \sum_{n=1}^{N-1} \Delta(\Delta\Gamma_n^{\ddagger}), \tag{22a}$$

where the first balanced position is close to the binding center as it was already mentioned after formula (2b), N is the ligand balanced positions number inside the channel. If the channel always expands from the binding center, then $\Delta(\Delta\Gamma_n^{\ddagger}) = \Delta(\Delta\Gamma_n^{\ddagger *})$ and according to formula (22a) we have:

$$\Delta\Gamma_p = \Delta\Gamma_p^* > 0, \quad \Delta\Gamma_p^* \equiv \sum_{n=1}^{N-1} \Delta(\Delta\Gamma_n^{\ddagger *}). \tag{22b}$$

When the channel always narrows from the binding center, then according to our both systems of numeration and formula (15) we get $\Delta(\Delta\Gamma_n^{\ddagger}) = -\Delta(\Delta\Gamma_{N-n}^{\ddagger *})$ and thus according to formula (22a) and the definition in formula (22b) we have:

$$\Delta\Gamma_p = -\Delta\Gamma_p^* < 0. \tag{22c}$$

The number of ligand balanced positions inside of a protein access channel may exceed 7 [7, 10, 17]. It means that the difference of the drag pseudopotentials on the ends of the whole channel may be considerable. According to (21), (22b) and (22c) we have:

$$\sup\left\{\frac{|\Delta\Gamma_p|}{R\cdot T}\right\} \approx 0.26. \tag{22d}$$

As it was already mentioned, the binding constant usually is determined by formula (2a) under the conditions of equilibrium and zero average velocities of the ligand translocations to and out of the binding center [20]. But due to the restriction of the number of ligand possible trajectories by an access channel, the second condition is not met. To correct (2a) let us take into account formulas (2c) and (22a). Thus we can write:

$$K_{binr} \approx K_{bin}\cdot\psi, \quad \psi \equiv \exp\left(\frac{\Delta\Gamma_p}{R\cdot T}\right), \tag{23a}$$

where K_{binr} is the real binding constant, while K_{bin} is the one calculated according to (2a).

According to (23a) and (22d) the real binding constant may be considerably different than the calculated according to formula (2a), so we get:

$$\inf\{\psi\} \approx 0.8, \quad \sup\{\psi\} \approx 1.3. \tag{23b}$$

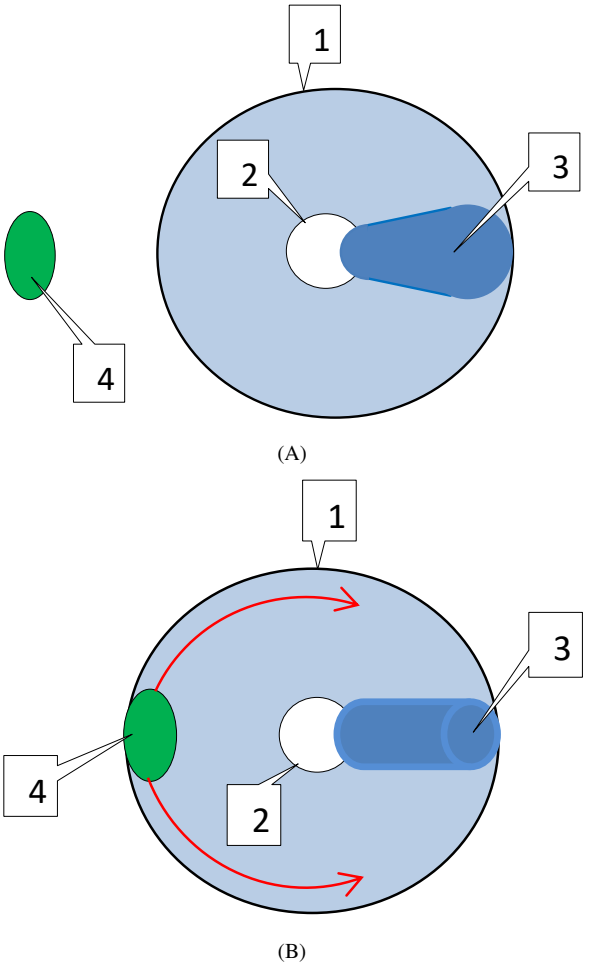

Fig. 4: Schematic representation of possible changing of a protein access channel shape by a binding effector. A – the binding effector is free, B – the binding effector is bound by the protein. 1 is the protein globular molecule, 2 is the binding center, 3 is the protein access channel, 4 is the effector molecule. Red arrows mark the protein intramolecular densification.

III. Analysis of the model for some natural applications

It is known that all binding effectors impact the conformational state of a protein [4, 21]. In such a way the Gibbs free energies difference ΔG in formula (2c) changes. Thus, the binding effectors influence the binding constant. But they may also change an access channel shape. In other words, they can change ψ in formula (23a). Thus, binding near the protein surface the effectors cause densification of outer protein molecule layer (Fig. 4).

In such a way they geometrically may decrease the value of a in formula (14a). Most protein access channels expand from the buried binding center [24]. According to (20a) – (23b) the changing of a exactly from 0.1 to 0 may decrease the ligand binding constant 1.3 times. It may be quite noticeable, for example, regarding oxygen release processes from hemoglobin to tissues or from myoglobin to mitochondria in hypoxic conditions [2–5].

As it was mentioned, the process of a ligand binding by a protein is not only used for carrying different metabolites through an organism. This process also is the base of almost all biological catalytic reactions [1]. In the latter case a binding center becomes as a catalytic center, where substrates transform to products and vice versa through an intermediate molecule:

$$\begin{aligned} N_{s1}S_1 + N_{s2}S_2 + \ldots + N_{sn}S_n + F \rightleftharpoons inter \cdot F \\ \rightleftharpoons N_{p1}P_1 + N_{p2}P_2 + \ldots + N_{pm}P_m + F, \end{aligned} \tag{24}$$

where S_i is i-th substrate, F is an enzyme, P_j is j-th product, N_{si} and N_{pj} are stoichiometric numbers for substrates and products respectively, $inter \cdot F$ is the complex of the intermediate with the enzyme in its catalytic center, n and m are the substrates and products number correspondingly. The equilibrium constant of reaction (24) K_{eq} equals the relation of the binding constants for the substrates K_{binsub} and products K_{binpro} within the protein correspondingly:

$$\begin{aligned} K_{eq} &\equiv \frac{[P_1]^{N_{p1}} \cdot [P_2]^{N_{p2}} \cdots [P_m]^{N_{pm}}}{[S_1]^{N_{s1}} \cdot [S_2]^{N_{s2}} \cdots [S_n]^{N_{sn}}} = \frac{K_{binsub}}{K_{binpro}}, \\ K_{binsub} &= \frac{[inter \cdot F]}{[S_1]^{N_{s1}} \cdot [S_2]^{N_{s2}} \cdots [S_n]^{N_{sn}} \cdot [F]}, \\ K_{binpro} &= \frac{[inter \cdot F]}{[P_1]^{N_{p1}} \cdot [P_2]^{N_{p2}} \cdots [P_m]^{N_{pm}} \cdot [F]}, \end{aligned} \tag{25}$$

where $[S_i]$, $[P_j]$, $[F]$ and $[inter \cdot F]$ are equilibrial concentrations of i-th substrate, j-th product, enzyme and the complex of the enzyme with the intermediate, correspondingly. According to (23a) and (25) for a monomolecular reaction with $n = m = 1$ in (24) we have:

$$K_{eqr} = K_{eq} \cdot \psi_{eq}, \quad \psi_{eq} \equiv \exp\left(\frac{\Delta\Gamma_{ps} - \Delta\Gamma_{pp}}{R \cdot T}\right), \tag{26}$$

where K_{eqr} is the real equilibrium constant, K_{eq} is the equilibrium constant calculated according to formulas (2a) and (25), $\Delta\Gamma_{ps}$ and $\Delta\Gamma_{pp}$ are the differences of the drag pseudopotentials on the ends of the whole channel (or different channels) for the substrate and product, correspondingly.

Let us consider the case when the substrate and product of such a reaction get to the active center via only one access channel (Fig. 5, top row). In such a situation, according to formula (22b) or (22c) and the written before them, the signs of $\Delta\Gamma_{ps}$ and $\Delta\Gamma_{pp}$ are the same.

Taking into account that the substrate and product diameters are close to each other, according to (18) and (22a) we have $\Delta\Gamma_{ps} \sim \Delta\Gamma_{pp}$. Thus according to formula (26) the equilibrium constant of a monomolecular reaction catalyzed by an enzyme with one access channel is almost independent from the channel shape.

Now let us consider the case when the substrate and product of a monomolecular reaction get to the active center via different access channels, the first of which narrows ($a_s > 0$), while the second expands ($a_p < 0$) with the approach to the active center (Fig. 5, bottom row) or vice versa. According to formula (22b) or (22c) and the written before them, the signs of $\Delta\Gamma_{ps}$ and $\Delta\Gamma_{pp}$ differ.

Let us consider the case, when the channels have slight gradient of their inner diameters: $|a| \approx 0.1$ and are long enough so that the ligand molecule inside them have approximately 7 balanced positions. In the active center the channels diameters should be close to one another. In such a case the smallest inner diameter of the wider channel is considerably bigger than the smallest inner diameter of the narrower one. Therefore, according to (19c) – (20g), (22a) – (22c) and (26) a contribution of the wider channel is neglectable. Thus analogically to (23b) we have:

$$\inf\{\psi_{eq}\} \approx 0.8, \quad \sup\{\psi_{eq}\} \approx 1.3. \tag{27}$$

According to (27) the real equilibrium constant of a monomolecular reaction catalyzed by an enzyme having different access channels for substrate and product may considerably depend on the channels shape.

Let us consider the case when a biochemical reaction with $n > 1$ or $m > 1$ in (24) is catalyzed by a ferment with two different access channels for substrates and products, one of which narrows, while the other expands with the approach to the active center or vice versa. For such a reaction more than one ligand molecule should be translocated through the channels. It leads to multiplication of the drag pseudopotentials differences located in the exponent of formula (26). The real equilibrium constant of such a reaction may be shifted even more than monomolecular one:

$$\inf\{\psi_{eq}\} \approx 0.8^k, \quad \sup\{\psi_{eq}\} \approx 1.3^k, \tag{28}$$

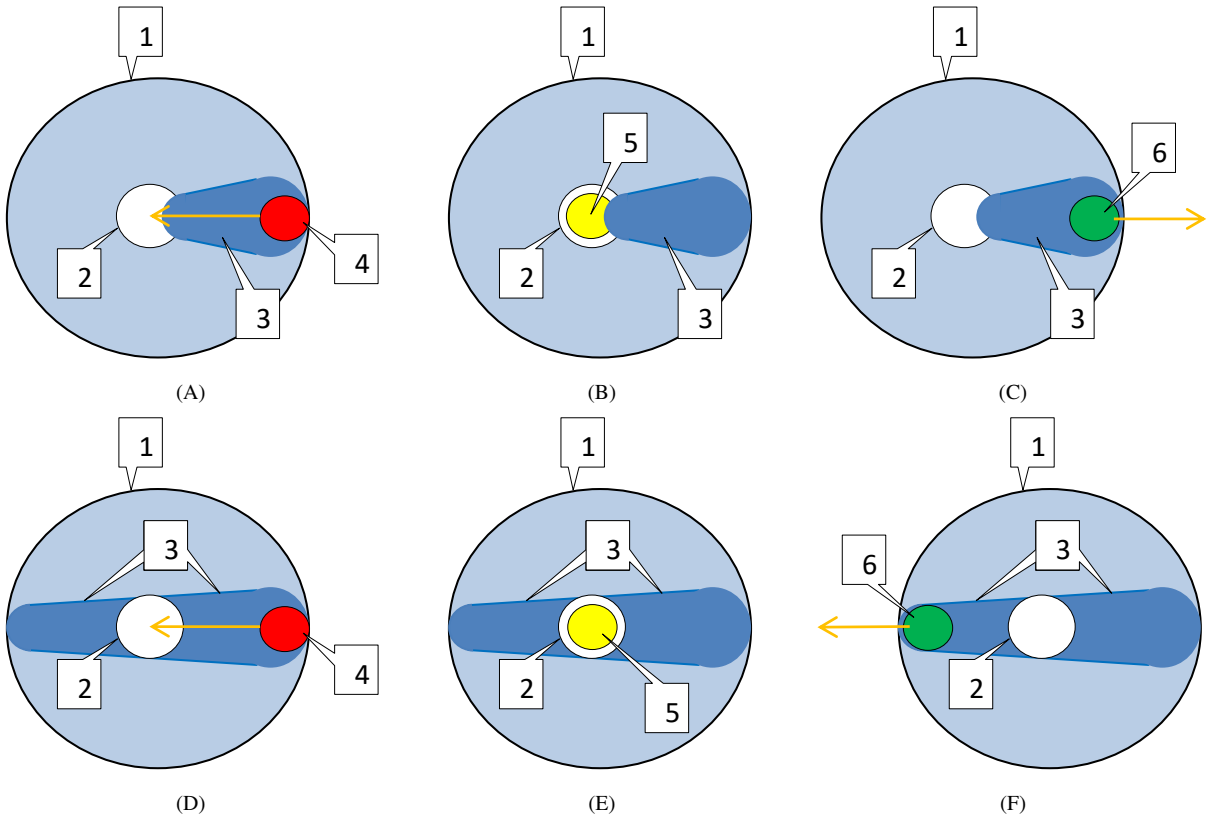

Fig. 5: Schematic representation of an enzymatic monomolecular reaction. A, B, C are different stages of the reaction, which is catalyzed by an enzyme with the only access channel for the substrate and product. D, E, F are different stages of the reaction, which is catalyzed by a ferment with two different access channels. 1 is the protein globular molecule, 2 is the catalytic center, 3 are the protein access channels, 4 is the substrate molecule, 5 is the intermediate, 6 is the product.

where k equals n in the case when the substrate access channel is narrower, or m when the product channel is narrower.

As it was already mentioned, intramolecular channels of proteins are widely used by living cells also for rapid transmembrane transport of different metabolites [15]. As it is known, L-type of Ca^{2+}-channels considerably expand in the direction to the membrane surfaces [14] (Fig. 6). According to our model it leads to excess (near 1.3-fold) accumulation of Ca^{2+}-ions in the middle of the channel, in comparison with the described by Eisenberg [25]. This can additionally increase Ca^{2+}-affinity of the channel.

Due to the demonstrated gradients of diameters of transmembrane channels [17] and according to (19a) – (22a), in the state of dynamic equilibrium they can create additional transmembrane electrochemical potential difference μ_{ad} (note that the same applies to pumps, which also use access channels [15]):

$$|\Delta\mu_{ad}| = |\Delta\Gamma_p|. \tag{29}$$

In conditions of room temperature according to (22d) and (29) we have:

$$\sup\{|\Delta\mu_{ad}|\} = \sup\{|\Delta\Gamma_p|\} \sim 670\ J/mol. \tag{30}$$

According to (30), possible excess transmembrane electrochemical potential difference created by biological ion channels and pumps with inner diameter gradient is much smaller in comparison with physiological transmembrane electrochemical potential difference [1]. It means that such a phenomenon does not play considerable role in cell charging.

Analogically, we can say the same about the shifting of a biochemical reaction (24) right or left due to the access channels inner gradients. It cannot be used in ATP or other macroergic compounds accumulation because $|\Delta G|$ of their synthesis is of order 30 kJ/mol

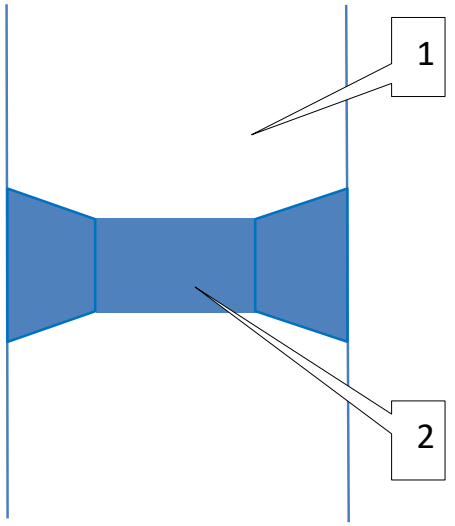

Fig. 6: Schematic representation of the L-type Ca^{2+}-channel structure. 1 is the phospholipid bilayer membrane, 2 is the Ca^{2+}-channel.

[1]. It is considerably higher than 670 J/mol, which is calculated in our model according to formula (30).

Formula (4) connects the work, which should be done by fluctuation force I during the ligand binding by a protein, with the work of the drag force, and thus with the drag pseudopotential difference on the ends of the protein access channel, calculated in our model by means of formulas (18) – (22d). Thus, in the framework of our model, it was shown that due to the fluctuation force (or the thermal energy) the equilibrium between free and binded ligand by a protein may be considerably shifted. Analogically, the equilibrium between the substrates and products of a biochemical reaction may be shifted, or a metabolite may be accumulated unevenly on both sides of the membrane.

Thus, it may become another variety of thermal energy harvesting. Presently, the thermal energy harvesting is known mainly as thermoelectric conversion of thermal energy into electrical one, due to new technological possibilities to control of phonon generation, relaxation and propagation in the nanoscale [26]. Proteins are also nanoscale devices [1, 6]. As it was shown in this article, protein molecules may also harvest thermal energy in small amounts, due to their access channels diameter gradients. Such type of thermal energy harvesting may play certain role in functioning of living organisms. This role should be studied further.

IV. Conclusion

1) A ligand inside of a protein access channel should dissipate considerable amount of energy for overcoming the activation barrier. Such an energy value is called a drag barrier of activation.
2) The drag barrier of activation is proportional to the temperature and to the squared ratio of the ligand diameter and the distance-averaged channel diameter.
3) The drag barriers of activation for the forward and backward ligand equilibrial translocations may differ inside of an access channel which has an inner diameter gradient.
4) Such differences may be considerable. They exponentially have an effect on the binding constant.

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