

Study of Functional Relevant Conformational Mobility of β -2 Adrenoreceptor by Means of Molecular Dynamics Simulation

G. V. Novikov, V. S. Sivozhelezov, S. S. Kolesnikov, K. V. Shaitan

Abstract—The study reports about the influence of binding of orthosteric ligands as well as point mutations on the conformational dynamics of β -2-adrenoreceptor. Using molecular dynamics simulation we found that there was a little fraction of active states of the receptor in its apo (ligand free) ensemble corresponded to its constitutive activity. Analysis of MD trajectories indicated that such spontaneous activation of the receptor is accompanied by the motion in intracellular part of its alpha-helices. Thus receptor's constitutive activity directly results from its conformational dynamics. On the other hand the binding of a full agonist resulted in a significant shift of the initial equilibrium towards its active state. Finally, the binding of the inverse agonist stabilized the receptor in its inactive state. It is likely that the binding of inverse agonists might be a universal way of constitutive activity inhibition *in vivo*. Our results indicate that ligand binding redistribute pre-existing conformational degrees of freedom (in accordance to the Monod-Wyman-Changeux-Model) of the receptor rather than cause induced fit in it. Therefore, the ensemble of biologically relevant receptor conformations is encoded in its spatial structure, and individual conformations from that ensemble might be used by the cell in conformity with the physiological behavior.

Keywords—Seven-transmembrane receptors, constitutive activity, activation, x-ray crystallography, principal component analysis, molecular dynamics simulation.

I. INTRODUCTION

SEVEN-TRANSMEMBRANE (7-TM) receptors belong to a large class of signaling proteins involved in cellular responses to a variety of extracellular signals, such as hormones and neurotransmitters, olfactory substances, pheromones and even light stimulation. Thus 7-TM receptors have become main targets for many pharmaceutical drugs. Recently, it became clear that 7-TM receptors are proteins with significant structural dynamics due to their ability to form different conformations *in vivo* depending on external conditions. On Fig. 1 the modern concept of receptor functional efficacy is shown according to which binding of different ligands stabilize receptor in different conformation regions which are associated with its different functional activity in context of cell.

G.V. Novikov is with the Institute of Cell Biophysics, 142290, Pushchino, Russia (phone: +79152620379; e-mail: gnovikov87@gmail.com).
V. S. Sivozhelezov and S. S. Kolesnikov are with the Institute of Cell Biophysics, 142290, Pushchino, Russia.

K. V. Shaitan is with the Lomonosov Moscow State University, Moscow, Russia (e-mail: k.v.shaitan@molsim.org).

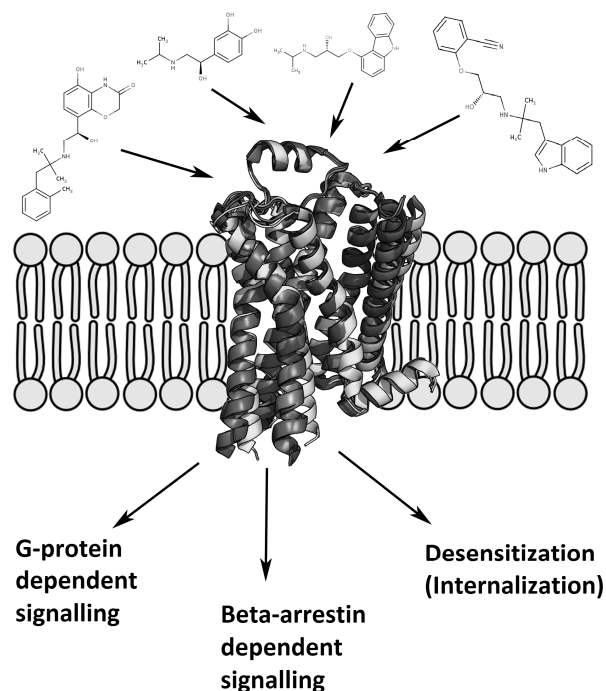


Fig. 1 “Functional selectivity” of the β -2-adrenoreceptor

However current ideas of the receptor activation still lack complete clarity. For example, there is a body of experimental data indicating that full agonist binding does not always result in stabilization of the receptors active state [1]. At the same time, one of the important functionally relevant properties of ligand-activated receptors is the existence of their constitutive (ligand-independent) activity, which has been detected *in vivo* for many rhodopsin-like receptors [2]. Taking into account a huge number of diseases produced by the constitutively active receptors, the cell has to play the role of a rheostat, intended to minimize the number of spontaneously activated receptors. Therefore, qualitative description of receptor conformations corresponding to its true active states is needed, as well as the exact routes in the conformation space of the receptor, sampled while achieving those states. According to the modern concept a functional receptor should be considered as an ensemble of isoenergetic conformers. Within this notion the influence of any external factors (such as the binding of orthosteric ligands or allosteric modulators, changes in the pH level or salt concentration) must result in redistribution of the pre-existing conformational space, stabilizing its specified

regions. In accordance with [3] each receptor's functionally relevant sub-state should also be considered as a sub-ensemble of thermally accessible conformations. Within this concept, the central task of modern drug design is the development of ligands which would stabilize the narrowest regions of the conformational space of receptors by binding, thereby, excluding receptor conformations associated with undesirable side effects. This data suggest that receptor's activation should be envisaged from the point of view of its conformational dynamics within the theory of statistical ensembles as well as the concept of free-energy surfaces. Within this concept the search for methods which would allow obtaining explicit representation of the full range of the accessible sub-states of receptors is also very relevant. Based on these assumptions we used molecular dynamics simulation, as well as methods of structural bioinformatics, to investigate the process of receptor activation. By means of this complex approach we investigated the influence of ligand binding as well as the effect of specific point mutation insertions on the pre-existing conformational equilibrium of β -2-adrenoreceptor. Our results agree with the fundamental theoretical models which describe receptors functional behavior [3], [4] as well as with up-to-date experimental data [5], [6]. At the same time we propose method for qualitative estimation of the receptors conformational equilibrium *in silico*. It is known that undesirable side effects detected *in vivo* also are associated with the stabilization of some receptor sub-states. Thus besides fundamental significance our finding might have applied engineering in drug design.

II. METHODS

A. Method of Principal Component Analysis

The method of principal component analysis (PCA) — is one of the major techniques of decrease of master data dimensions, with a minimal loss of significant information [7], [8]. Normally, the calculation of principal components (PC) is reduced to the calculation of eigenvectors and eigenvalues of covariance matrix of master data. According to the general idea of this approach, the main directions of the most collective fluctuations (i.e., synchronous oscillations of a large number of atoms), making the biggest contribution to the observed conformation mobility of proteins.

Conformational dynamics, observed along separate PC, are obtained from conformer ensembles of a studied protein. If we consider a trajectory containing N atoms $F(X_i, Y_i, Z_i)$, where $i = 1, 2, \dots, N$. The first step in the analysis of experimental structure ensembles includes the calculation of covariance matrix C , consisting of $3N \times 3N$ elements (1):

$$\langle \Delta R \Delta R^T \rangle = m^{-1} \sum_A [\Delta R^{(A)} \Delta R^{(A)T}] \quad (1)$$

R in (1) is a set of coordinates for a point in trajectory; all the summation is realized for all the analyzed structures m , while ΔR is a deviation indicator for a certain function A , relative to the average structure $\langle R \rangle$. The index T denotes

transpose of the initial matrix. Subsequent diagonalization of the matrix C , using (2):

$$C = \sum_{i=1}^m \sigma_i p_i p_i^T \quad (2)$$

results in the calculation of principal components (eigenvectors) p_i , as well as corresponding dispersion values (eigenvalues) σ_i . In this equation, σ_i represent the ultimate dispersion of the system, while p_i ($3N$ -dimensional vector) describes the bias of N -bases, represented in this context with their C - α -atoms, according to this most variable mode, which is termed as the first principal component. Finally, root-mean-square deviation between the analyzed structures can be calculated by summation of diagonal elements of matrix C , according to (3):

$$\langle RMSD \rangle = [tr(C) / N]^{1/2} \quad (3)$$

B. Method of Molecular Dynamics Simulation

The method of molecular dynamics (MD) is based on computational solution of classical Newton's equations of motion for a polyatomic system. Within this method, all atoms of a given system represent material points, and the behavior of a single atom is described by the classical motion equations. Since the practical studies are concerned about finite length trajectories, the large-scale conformational movements in proteins are commonly studied using the methods of steered molecular dynamics. Essential Dynamics Sampling (EDS) is one of such methods [9]. Since the conformation movements of macromolecules contribute significantly to the covariant fluctuations in the positions of individual atoms, the method of principal components is an effective way of MD trajectory analysis. The results of diagonalization of covariance matrix (eigenvector sets) can be applied for limiting the conformation mobility of a macromolecule in selected degrees of freedom, when making subsequent simulations. The set of employed eigenvectors (termed, in the context of the applied method, as an essential subspace) was formed by the first three low-frequency modes, calculated by the PCA method for the experimental set of β -2-adrenoreceptor. Earlier we have shown, that analysis of experimental sets of ligand-activated receptors, with the first low-frequency modes, exposes the most functionally significant movements in these proteins [10]. Experimental set up was carried out using Gromacs software package, applying Gromos 54A7 force field (the united-atom model) as well as Amber 99sb (full atomic model) with Berger's (united-atom) lipids parameters in both cases. The initial spatial model of β -2-adrenoreceptor was created based on its experimental structure, which corresponds to inactive state of the protein (pdb id 2rh1). Two extra systems were created for modeling β -2-adrenoreceptor in complexes with ligands (systems 2 and 3). Parameterization of a full agonist (isoprenaline), as well as an inverse agonist (ICI 118,551) was done on the ATB server (<http://compbio.biosci.uq.edu.au/atb/index.py>), using the algorithm PM3. The applied spatial conformations of ligands,

and their precise disposition in the orthosteric pocket of the receptor, corresponded to x-ray structural data in both cases (pdb ids 2y03, 3ny8). Fig. 2 exhibits the models for β -2-adrenoreceptor in complexes with full agonist (system 2), isoprenaline (Fig. 2 (a)), as well as with inverse agonist (system 3) ICI 118,551 (Fig. 2(b)).

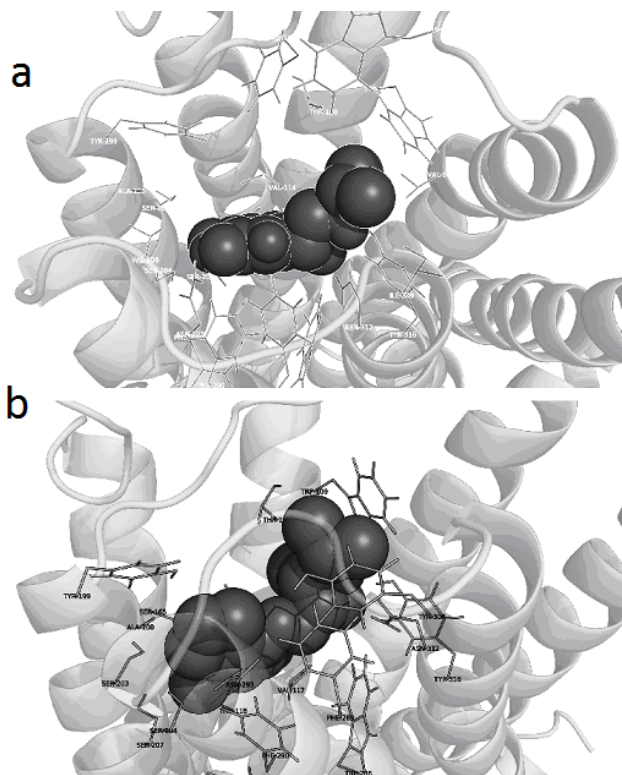


Fig. 2 (a) The models of β -2-adrenoreceptor in complex with full-agonist isoprenaline (up); (b) the model of this protein in complex with inverse agonist ICI 118,551. Both cartoon diagrams represent receptor conformation and the sticks corresponds to the side-chains involved in the indirect interaction with the functional groups of the ligands

Two additional models for β -2-adrenoreceptor were created to analyze the influence of point mutations on conformational dynamics of this protein, with the introduction of a number of point mutations in the different regions of its α -helix bundle (systems 4 and 5). [11]. Using Rotamer module of Chimera software, we introduced both sets of mutations into the inactive conformation of the receptor (model based on crystallographic structure 2rh1) in such a way; that system 4 corresponded to the potentially inactive mutant, while system 5 imitated the constitutively active mutant (CAM). Cartoon model in Fig. 3 shows the initial spatial structures of both mutated receptors.

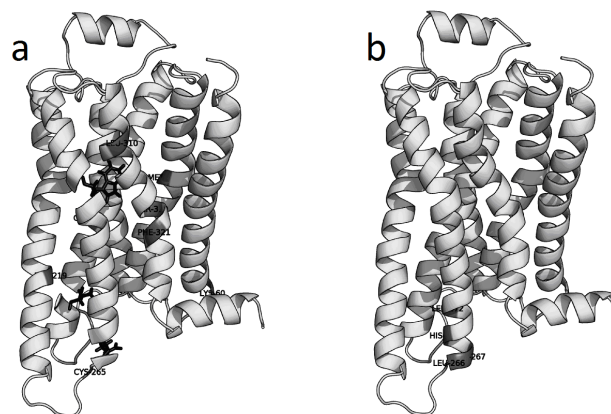


Fig. 3 Two sets of point mutations shown on the cartoon representation of β -2-adrenoreceptor (a) set of “inactivated” mutations is highlighted by sticks; (b) set of mutations is located in the intracellular end on the IV alpha-helix (marked by dark color)

On Fig. 3 (a) the set of “inactivating” mutations (system 4) are shown in a darker color. In the latter case, noteworthy is the spatial area of the mutated residues relative to the system of highly conservative residues (microswitchers, which are shown as sticks in the Fig. 3). Marked regions on the Fig. 3 (b) highlights the mutations, introduced into the amino acid residues of the cytoplasmic terminus of the sixth α -helix of the receptor (system 5). The summarized simulation protocol for all systems is given in Table I. Subsequently, the method of essential dynamics sampling was used for calculations of 100 ns MD trajectories of all five systems.

TABLE I
 PROTOCOL OF MOLECULAR DYNAMICS SIMULATION

Integrator	SD (Langeven's stochastic dynamics)
Timestep	2 fs
Nlist	5 fs
Calculation of the interatom interactions	grid
Periodic boundary conditions	xyz
Cutoff distance for the long-range interactions (rlist)	1.2 nm
Algorithm of columbic interactions calculation	PME (Particle Mesh Ewald)
Cut-off radius	1.2 nm
Van der Waals interactions	Cut-off
Cut-off radius	1.2 nm
Constraints	All bounds (LINCS)
Constant temperature	Constant temperature from the stochastic dynamics with relaxation time of kinetic energy ($\tau_t = 2$ ps).
Constant pressure	Modeling in NPT ensemble with the Parrinello-Rahman barostat using semiisotropic scaling (constant pressure 1 atm, independent scaling of x-y and z dimensions).

III. RESULTS

In one of the previous works we have studied functionally relevant structural movements of rhodopsin-like receptors, with reference to experimental sets of these proteins [10]. In present study, at first step we studied the conformational mobility of the apo form (ligand-free) of β -2-adrenoreceptor. According to the modern concept, the spatial architecture of

the receptor includes, along with regions of higher stability (transmembrane helices), more structurally flexible loop regions. Consequently these intrinsically disordered regions have the main contribution to the formation of allosteric properties of the receptor ensemble (entire spectrum of possible sub-states) [12], [13]. Functionally, the most conformationally labile regions in these proteins are responsible for binding with a wide variety of different extra- and intracellular partners. Hence, the advantage of computational simulations is in obtaining a detailed view on such macromolecule conformational dynamics at the full atomic level. On Fig. 4 (a) the projection of the calculated MD trajectory for the apo receptor (system 1) on the surface of principal components 1 and 3 of the main subspace is present.

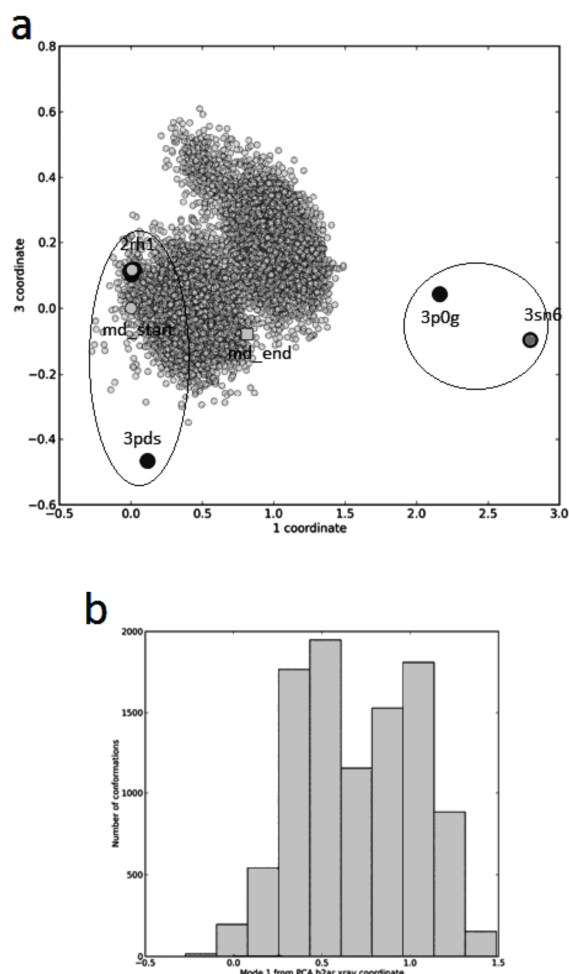


Fig. 4 (a) The superimposition of the snapshots of the molecular dynamics trajectory of apo β -2-adrenoreceptor (small light dots), as well as X-ray structures of that protein (big dots) projected on the plane of the essential subspace corresponded to the principal components 1 and 3 (PC 1 and PC3); (b) projection of the snapshots from that trajectory onto the surface of the PC-1

Analysis of such plots allows following the receptor “movement” in its conformation space, using X-ray structures of the protein as “reference points”. This plot clearly shows that over a modeling period of 100 ns apo β -2-adrenoreceptor

was spontaneously moving towards its active sub-state (right cluster of experimental structures). Therewith, the projection of the MD trajectory observed a bimodal distribution of the receptor conformers (Fig. 4 (b)), with the maximal density in the two areas of its conformational space. One of the sub-states was structurally identical to the initial point of simulation (x-ray structure pdb id 2rh1), while the other one had the typical attributes of activation of rhodopsin-like 7-TM receptors (2-2.5 Å chattering of intracellular terminus of helix VI). These data directly indicate the correlation between the structural mobility of ligand-activated receptors and their constitutive activity registered *in vivo*. Simulations of β -2-adrenoreceptor complexes with full and partial agonists (systems 2 and 3, respectively) were pursued to investigate the influence of orthosteric ligands on the structural properties of the protein. Dark dots in Fig. 5 (a) represent the projection of the obtained MD trajectory for the complex of the receptor with a full agonist on the plane of PC 1 and 3.

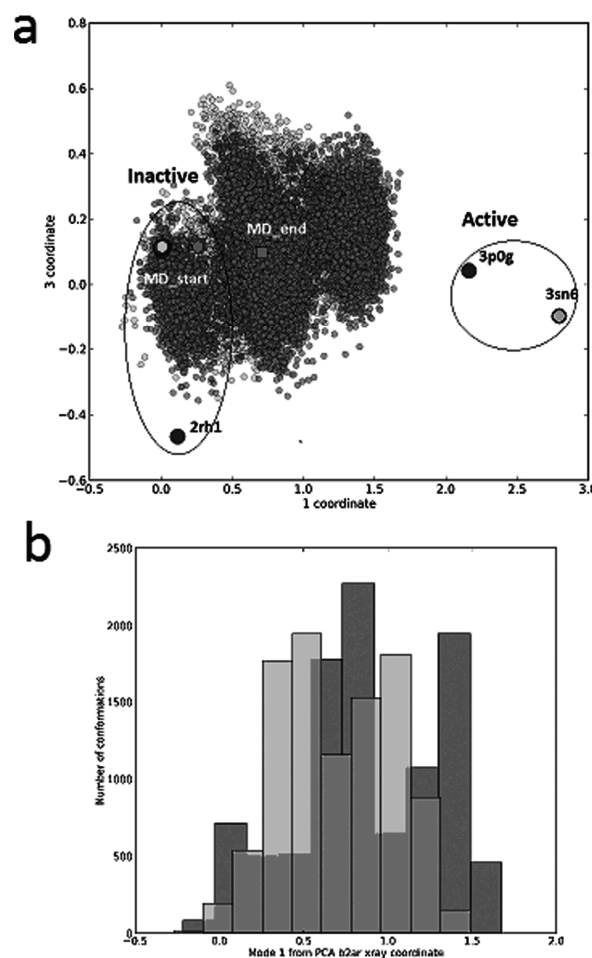


Fig. 5 (a) The superimposition of the snapshots of the molecular dynamics trajectories of apo β -2-adrenoreceptor (small light dots), complex of that protein with the full agonist (small dark dots) as well as X-ray structures of that protein (big dots) projected on the surface of the essential subspace corresponded to the principal components 1 and 3 (PC 1 and PC3); (b) the projection of the snapshots from both trajectories onto the surface of the PC-1 only

Light-colored dots on the plot correspond to the MD trajectory projection calculated for apo receptor. It appears from the diagrams that the presence of a full agonist in the orthosteric pocket of the receptor leads to a significant redistribution of density of its conformers, in comparison to its apo form. The pattern of the resulting distribution shows, that the binding of this ligand shifts the equilibrium to the active receptor sub-state in contrast to its apo form. As seen from Fig. 5 (b), the presence of full agonist results in higher stabilization of the intermediate sub-state of the receptor, not excluding the conformations, which correspond to the inactive sub-state, from the ensemble. The latter observation is also confirmed by the data of x-ray structure analysis, an approach used for resolving the spatial structure of β -2-adrenoreceptor in an inactive sub-state, as a complex with a full agonist (pdb id 3eml). Fig. 6 contains the chart of root-mean-square fluctuations for each amino acid residue in the receptor, as calculated using the trajectories of system 1 (apo receptor, light graph), as well as system 2 (complex of receptor with a full agonist, dark graph).

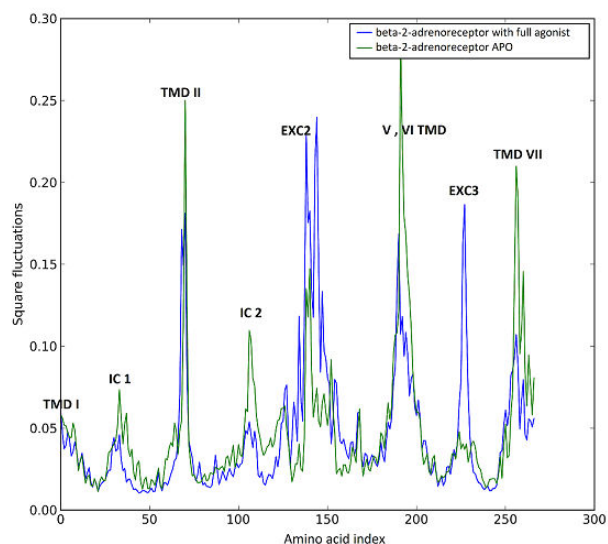


Fig. 6 Comparison of the root-mean squared fluctuations (RMSF) for each amino acid residue of the β -2-adrenoreceptor. Conformational mobility of the β -2-adrenoreceptor in case of its apo form and complex with full agonist

In the diagram it is seen that in both cases the receptor displayed apparent dynamic behavior, in particular, in its cytoplasmic termini of helices V-VII. Specifically, the most significant structural differences in the receptor complexed with a full agonist were observed in the mobility of its second and third extracellular loops. This indicates the influence of ligand in the orthosteric pocket of the receptor on the mobility of its extracellular (ligand-recognizing) region. Similar patterns have been also observed previously for other 7-TM receptors, particularly as a result of analysis of the respective experimental datasets [14]-[17]. These data confirm the structural interplay between the distant receptor segments carrying out specific functions (for instance, ligand or

transducer binding). On the other hand, simulation of the receptor in a complex with a full agonist did not reveal any significant movements of the cytoplasmic termini of α -helices V and VI, registered in the x-ray structures of β -2-adrenoreceptor (amplitude of several Å), as well as rhodopsin (amplitude of ~ 6 Å). Analysis of MD trajectories for apo receptor and its complex with a full agonist detected only small fluctuations in the cytoplasmic termini of helices V and VI of the receptor (amplitude of ~ 2 Å). These intramolecular motions may lead to the opening-closing of the cytoplasmic region of the protein which can have functional significance for the interaction with different transducers. Interestingly, the receptor conformation with a more open cytoplasmic region was realized mostly in the complex with a full agonist. In other words, the binding of this ligand shifted the physicochemical equilibrium of the receptor towards its experimentally registered active conformations, augmenting the statistical weight of this sub-state in the preexisting ensemble. Dark dots on Fig. 7 (a) mark the projection of the calculated trajectory for the receptor complexed with an inverse agonist (system 3), on the surface of the main subspace, in respect to the trajectory of its apo form (light dots).

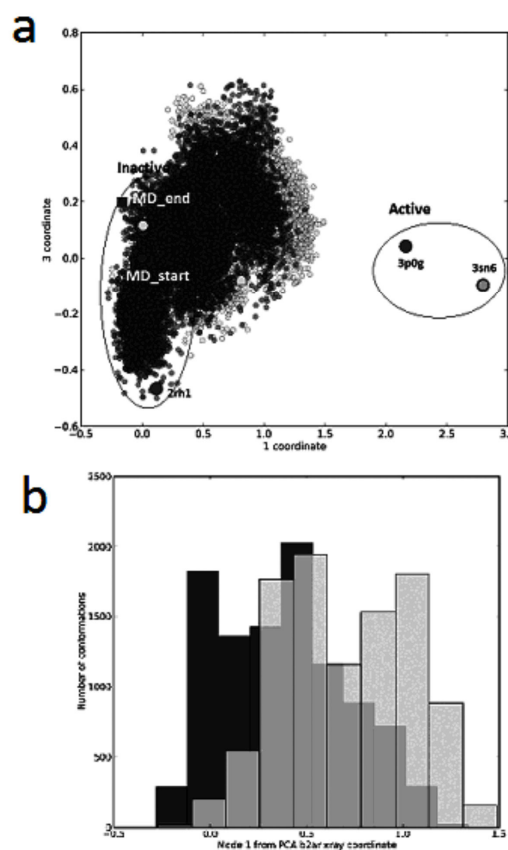


Fig. 7 (a) The superimposition of the snapshots of the molecular dynamics trajectories of apo β -2-adrenoreceptor (small light dots), complex of that protein with the inverse agonist (small black dots) as well as X-ray structures of that protein (big dots) projected on the surface of the essential subspace (PC 1 and PC3); (b) the projection of the snapshots from both trajectories onto the surface of the PC-1

The resulting distribution obviously indicates that the binding of this ligand results in significant shift of the physicochemical equilibrium of the receptor towards its inactive sub-state (Fig. 7 (b)). While modeling the receptor complexed with inverse agonist, it is important, that the ensemble lacks virtually all conformations present in the system with full agonist. Thus, the binding of an inverse agonist led to a significant increase of conformational rigidity, essentially limiting the area of its available conformational space. These observations are supported by the graph of root-mean-square deviations shown on Fig. 8, displaying less expressed fluctuations in the structurally flexible receptor regions.

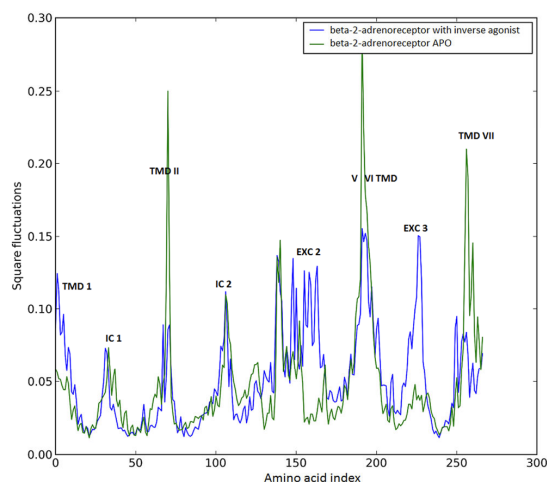


Fig. 8 Comparison of the root-mean squared fluctuations (RMSF) for each amino acid residue of the β -2-adrenoreceptor. That diagrams indicate conformational mobility of the β -2-adrenoreceptor in case of its apo form, as well as complex with inverse agonist

As seen from Fig. 8, inverse agonist binding resulted in only insignificant changes of mobility of the extracellular loops (amplitude of ~ 1.5 Å), which function as a vestibule into the orthosteric pocket of the receptor. At the same time, the mobility of receptor α -helices was much less expressed, in comparison to the other two models.

At the next step we tried to study the details of the correlation between receptor activation and the features of their spatial architecture. There is a plethora of experimental data where many of point mutations in β -2-adrenoreceptor have been described, which can shift the physicochemical equilibrium of the protein towards one of the biologically significant sub-states [18]. Therefore, the molecular dynamics approach is obviously applicable for evaluation of influence of these factors on the overall conformational dynamics of macromolecules (statistical distribution of conformers). Fig. 9 (a) illustrates a trajectory projection for the mutated receptor (dark dots) on the plane of principal components 1 and 3.

The statistical distribution shown of Fig. 9 (b) indicates that the functional efficacy of the mutated receptor is similar to the system, which was simulated in a complex with inverse agonist. Conversely, the model of β -2-adrenoreceptor with

mutations introduced into the cytoplasmic terminus of the sixth α -helix has a remarkable overall similarity with the system, simulated in a complex with a full agonist as can be seen on Fig. 10 (a).

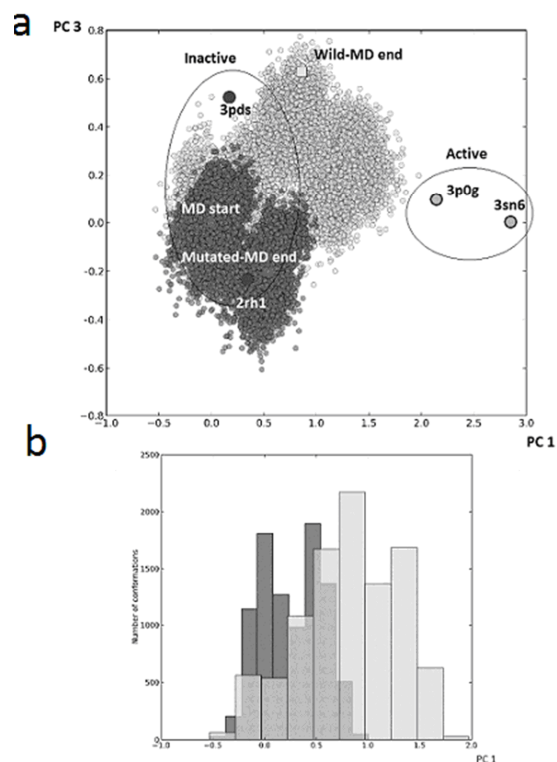


Fig. 9 (a) The projection of the snapshots extracted from the MD trajectory of the mutated receptor (black dots) as well as wild-type receptor (light dots) onto surface of the 1 and 3 principal components (b) the projection of the both trajectories onto surface of the 1 PC

Despite the absence of a characteristic three-peaks distribution in the resulting distribution pattern for the mutated receptor, which was registered upon simulation of a complex with a full agonist, in both cases shifting towards the active sub-state of the receptor was observed (Fig 10 (b)). On the other hand, the differences in conformer distribution along principal component 1 are indicative of the alteration in the transition to the active sub-state in both systems possessing the factors, which shift the equilibrium towards the active sub-state. This assumes the presence of several possible ways for transition of the receptor between two identical initial states, upon the influence of different external factors. At the same time, the similarity of performance of systems 3 and 5 in the presence of factors, stabilizing the inactive sub-state, suggests, that suppression of constitutive activity of ligand-activated receptors *in vivo* most likely conforms to a single robust mechanism selected in evolution.

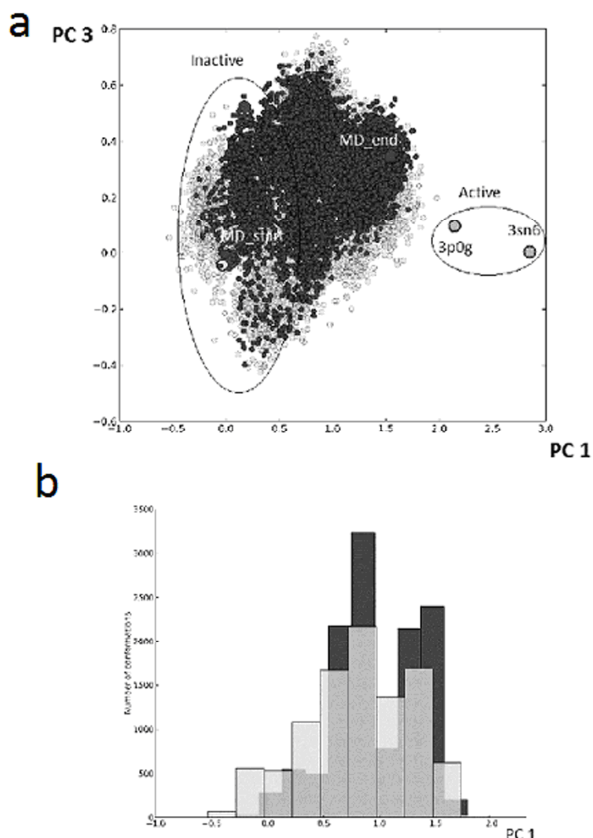


Fig. 10 (a) the projection of the MD snapshots of the mutated receptor (black) as well as wild-type receptor (light) onto surface of the 1-3 principal components: (b) the projection of the both trajectories onto surface of the 1 PC

The high resolution characteristics of the applied methods allowed carrying out a detailed study of the interrelation between the spontaneous functioning and structural features of the receptor. Rhodopsin-like receptors have a number of highly conserved residues, and the activity of their side groups, according to experimental data, is associated with activity of these proteins [19]. Therefore, spontaneous rearrangements of these intramolecular interactions in the receptor are connected with its functionally significant conformational dynamics. To test this possibility we performed 100 ns simulations for the distance between the charged side groups of Arg-131 and Glu-268 (ionic lock) in the receptor complexed with different agonists (systems 2 and 3). As seen from the Fig. 11a, the presence of a full agonist in the orthosteric receptor pocket resulted in absolute destabilization of the ion lock during the whole simulation time. By contrast, the polar contact between residues Arg-131 and Glu-268 was almost completely preserved in the system, including the receptor with an inverse agonist. Thus, the ion lock dynamics shown on Fig 11B is an intrinsic regulatory mechanism of conformational mobility in rhodopsin-like receptors in response to its interactions with different external factors.

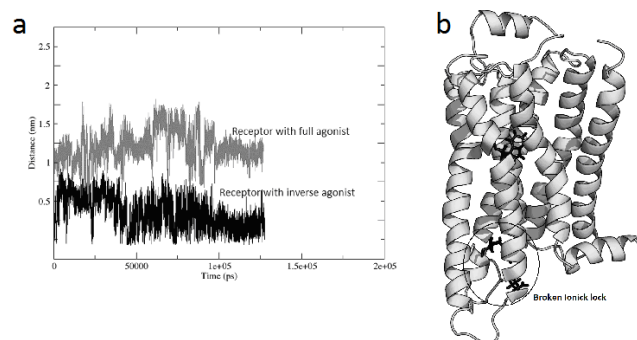


Fig. 11 (a) Dynamics of the ionic lock measured during 130 ns simulation in case of the receptor in complex with the full agonist (gray graph) as well as with inverse agonist (black graph). Values of the x-plane correspond to the time of simulation in picoseconds (ps) and on the y-plane correspond to the distance in nanometers (nm) between both polar side-chain groups of the ionic lock; (b) the cartoon diagram of the receptor with microswitches marked by sticks

IV. CONCLUSIONS

Analysis of molecular simulations indicates that ligand-activated receptors exhibit continuous walk in the areas of thermal-accessible conformational space, where the precise limits are predetermined from the fold patterns of these proteins. At the same time, interactions with various external factors result in stabilization of certain regions of the preexisting conformational space, which increases their statistical weight. Consequently, the ensemble of the possible sub-states of these proteins is predetermined at the level of its tertiary structure, and individual conformations can be used by the cell according to its physiological requirements.

ACKNOWLEDGMENT

This work was carried out with the financial support of Russian Foundation for Basic Research (grant No. 14-04-01198).

REFERENCES

- [1] Deupi X., Standfuss J. 2011. Structural Insights into Agonist-Induced Activation of G-Protein-Coupled Receptors. *Curr Opin Struct Biol.* 21, pp. 541-551.
- [2] Costa T., Cotecchia S. 2005. Historical Review: Negative Efficacy and the Constitutive Activity of G-Protein-Coupled Receptors. *Trends Pharmacol Sci.* 26, pp. 618-624.
- [3] Kenakin T. 2002. Efficacy at G-Protein-Coupled Receptors. *Nature Reviews Drug Discovery.* 1, pp. 103-110.
- [4] Canals M., Lane J. R., Wen A., Scammells P. J., Sexton P. M., Christopoulos A. 2012. A Monod-Wyman-Changeux Mechanism Can Explain G Protein-Coupled Receptor (GPCR) Allosteric Modulation. *J Biol Chem.* 287, pp. 650-659.
- [5] Nygaard R., Zou Y., Dror R. O., Mildorf T. J., Arlow D. H., Manglik A., Pan A. C., Liu C. W., Fung J. J., Bokoch M. P. 2013. The Dynamic Process of β -2-Adrenergic Receptor Activation. *Cell.* 152, pp. 532-542.
- [6] Kim T. H., Chung K. Y., Manglik A., Hansen A. L., Dror R. O., Mildorf T. J., Shaw D. E., Kobilka B. K., Prosser R. S. 2013. The Role of Ligands on the Equilibria between Functional States of a G Protein-Coupled Receptor. *J Am Chem Soc.* pp.
- [7] Ma S., Dai Y. 2011. Principal Component Analysis Based Methods in Bioinformatics Studies. *Brief Bioinform.* pp.

- [8] Hayward S., Kitao A., Go N. 1995. Harmonicity and Anharmonicity in Protein Dynamics: A Normal Mode Analysis and Principal Component Analysis. *Proteins*. 23, pp. 177-186.
- [9] Amadei A., Linssen A., De Groot B., Van Aalten D., Berendsen H. 1996. An Efficient Method for Sampling the Essential Subspace of Proteins. *Journal of Biomolecular Structure and Dynamics*. 13, pp. 615-625.
- [10] Novikov G., Sivozhelezov V., Shaitan K. 2013. Study of Structural Dynamics of Ligand-Activated Membrane Receptors by Means of Principal Component Analysis. *Biochemistry (Moscow)*. 78, pp. 403-411.
- [11] Javitch J. A., Fu D., Liapakis G., Chen J. 1997. Constitutive Activation of the β_2 Adrenergic Receptor Alters the Orientation of Its Sixth Membrane-Spanning Segment. *Journal of Biological Chemistry*. 272, pp. 18546-18549.
- [12] Hilser V. J., Thompson E. B. 2007. Intrinsic Disorder as a Mechanism to Optimize Allosteric Coupling in Proteins. *Proceedings of the National Academy of Sciences*. 104, pp. 8311-8315.
- [13] Unal H., Karnik S. S. 2012. Domain Coupling in GPCRs: The Engine for Induced Conformational Changes. *Trends Pharmacol Sci*. 33, pp. 79-88.
- [14] Scarselli M., Li B., Kim S. K., Wess J. 2007. Multiple Residues in the Second Extracellular Loop are Critical for M3 Muscarinic Acetylcholine Receptor Activation. *J Biol Chem*. 282, pp. 7385-7396.
- [15] Wheatley M., Simms J., Hawtin S., Wesley V., Wootten D., Conner M., Lawson Z., Conner A., Baker A., Cashmore Y. 2007. Extracellular Loops and Ligand Binding to a Subfamily of Family A G-protein-Coupled Receptors. *Biochemical Society Transactions*. 35, pp. 717.
- [16] Bokoch M. P., Zou Y., Rasmussen S. G., Liu C. W., Nygaard R., Rosenbaum D. M., Fung J. J., Choi H. J., Thian F. S., Kobilka T. S., Puglisi J. D., Weis W. I., Pardo L., Prosser R. S., Mueller L., Kobilka B. K. 2010. Ligand-Specific Regulation of the Extracellular Surface of a G-Protein-Coupled Receptor. *Nature*. 463, pp. 108-112.
- [17] Peeters M., Van Westen G., Li Q., Ijzerman A. 2011. Importance of the Extracellular Loops in G Protein-Coupled Receptors for Ligand Recognition and Receptor Activation. *Trends Pharmacol Sci*. 32, pp. 35-42.
- [18] Pauwels P. J., Wurch T. 1998. Review: Amino Acid Domains Involved in Constitutive Activation of G-Protein-Coupled Receptors. *Molecular neurobiology*. 17, pp. 109-135.
- [19] Nygaard R., Frimurer T. M., Holst B., Rosenkilde M. M., Schwartz T. W. 2009. Ligand Binding and Micro-Switches in 7TM Receptor Structures. *Trends Pharmacol Sci*. 30, pp. 249-259.