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Molecular dynamics simulation of β-adrenoceptors and their coupled G proteins

Z.-Y. LI[1], C.-Y. SU[2], B. DING[1]

[1]Department of Pharmacy, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, China
[2]Department of Pharmacy, Jinan Dermatosis Prevention and Control Hospital, Jinan, China

Abstract. – OBJECTIVE: G protein-coupled receptors (GPCRs) constitute the largest membrane proteins superfamily. However, the interactions between them and the coupled heterotrimeric G proteins were little known. To get a deeper view of how the receptor bound to the G protein, we carried out the molecular dynamics' simulations of human Beta2 adrenoceptors (β1 and β2) and G protein (s and l) alpha subunit complexes by homology modeling.

MATERIALS AND METHODS: For homology modeling, the program modeller 9.11 was used with automodel module. Before dynamics simulation, the homology models were prepared by Protein Preparation Wizard module in Maestro 9.3. The Desmond program was used to perform molecular minimization and molecular dynamics simulation under OPLS-All atom 2005 force field with default parameters.

RESULTS: The results offered us the mechanism vividly in molecular level: (1) GPCR-G protein complex can be simulated without specific nanobody; (2) the G protein activation ability of GPCR can be explained by molecular dynamics simulation.

CONCLUSIONS: It is suggested that we could do molecular dynamics simulation of complex of GPCR-G protein without bound nanobody. Secondly, the simulation time reduced greatly by using homology modeling to generate complex of proteins. Thirdly, the molecular dynamics simulation will help us to know or even predict further protein-protein interactions.

Key Words:
GPCR, Homology modeling, Molecular dynamics simulation.

Introduction

The G protein-coupled receptors (GPCRs) were the largest superfamily in membrane proteins. They can be triggered by small hormones, peptides, and even light and activate the corresponding G proteins. Then, the downstream signaling cascades are turned on to exert their functions. Therefore, GPCRs play a crucial role as molecular switches and became one of the important areas of drug discovery[1]. The GPCR superfamily was classified into five main classes: rhodopsin, secretin, glutamate, adhesion, and frizzled-taste-2[1]. Despite their significance in physical activities and medical functions, little is known about their structures due to the difficulty in purification and crystallization of crystal structures. Until recent times, little structures belonging to the rhodopsin family and a crystal structure belonging to Class F were solved[3-6]. Since most of these structures are crystalized in receptor themselves, we cannot know the interaction between them and G proteins. Fortunately, in 2011, Kobilka group solved the complex structure of Beta2 (β2)-adrenoceptor and its coupled Gs protein, which is a milestone in GPCR structural biology[7-10]. This structure gives us a long-awaited knowledge about how GPCRs interact with Gs protein. In the same year, Schertler solved the structure of rhodopsin and a short peptide of Galpha protein[11], which also offers us a view of how receptor couples with a peptide of GalphaCT2 protein[12]. However, both crystal structures merely provide us a rigid view. At the same time, due to the complexity of crystallization, we cannot get more complex structures in a short time. To solve this problem, we used the homology modeling method to generate models of β adrenoceptors and their coupled G proteins. We did this because the crystal structure of Beta1 and 2 adrenoceptor bound with agonist and antagonist has been determined and the crystal structure of Beta2 adrenoceptor gives us a vivid view of the movement of the specific transmembrane alpha helix5 and helix 6, we can use our molecular dynamics simulation to verify it and then elucidate the mechanism at the atomic level.

So far, several papers[13,14] have been published associated with molecular dynamics simulation of an active state of Beta-adrenoceptor. However,

Corresponding Author: Bo Ding, MD; e-mail: 1471128187@qq.com

they only provided the existed results which had been elucidated by crystal structure or suggested the reason why we always got the inactive crystal structures which are not the active crystal structures even bound with agonist. Up to now, little simulations had been published associated with receptor-G protein interaction. Therefore, we wanted to get a deeper view of how the receptor bound to the G protein. Since all β adrenoceptors are linked to Gs protein and β2 adrenoceptor link to Gi protein, in this work, we have presented the molecular dynamics simulation of complexes of β1 adrenoceptor-Gs protein, β2 adrenoceptor-Gs protein, and β2 adrenoceptor-Gi protein.

Materials and Methods

Homology Modeling Section

The crystal structure of β2 adrenoceptor and Gs protein complex (Protein Data Bank identification (PDB ID): 3SN6[7]) was used as a template. The amino acids sequences of β1 adrenoceptor, β2 adrenoceptor, β3 adrenoceptor, Gs protein, and Gi protein came from Uni-Prot database with ID number P08588, P07550, P13945, Q5JWF2, and P63096, respectively. The amino acids sequence of the crystal structure of β2 adrenoceptor and Gs protein complex was obtained by modeller 9.11[15] alignment function. Alignment of template and target protein was performed by Cobalt server[16, 17]. For homology modeling, the program modeller 9.11[18] was used with automodel module.

Molecular Dynamics Simulation

Before dynamics simulation, homology models were prepared by the Protein Preparation Wizard module in Maestro 9.3. All hydrogen atoms were added and termini were capped. After the hydrophobic helixes of the models were coordinated with a POPC lipid bilayer, the complex models were put into an orthorhombic box with size 10.0Å×10.0Å×23.0Å which was then solvated with SPC water model and 0.15 M NaCl ions. The program that[19] was used to perform molecular minimization and molecular dynamics simulation under OPLS-All-atom 2005 force field[20] with default parameters. For each complex model, a 5 ns simulation in the NPT ensemble was performed.

Results

Modeling the Active State of Beta Adrenoceptor-Gs Complex

It is not feasible to make a simulation of GPCR in a persistent active state by just modeling with a crystalized structure in active state. This is because agonist alone cannot stabilize the GPCR in active state. In a long time, it was thought to be impossible to crystalize GPCR in active state. A canonical E/DRY domain forms an ionic lock which makes the inactive GPCR stable[21]. That's why we always got GPCRs in inactive state. Fortunately, Kobilka invented a method to use nanobody to stabilize active Beta2 Adrenoceptor. So, to perform the simulations of the active state structures of Beta Adrenoceptor in a persistent long time, we used the camel-nanobody bound Beta2 adrenoceptor structure together with a Gs protein[7] (PDBID: 3SN6) as a template to generate structure by homology modeling method. The nanobody can stabilize the active state of Beta adrenoceptor but here in the homology models we deleted it because we wanted to see models moved in a natural state without artificial restraint. In the meantime, we thought we could still also obtain the active Beta-adrenoceptor because we kept the Alpha subunit of G protein in the original place, which was inserted into the intracellular cavity formed by outward TM5 and TM6. At the same time, we deleted the Beta and Gamma subunit of G protein. This is due to the fact that the crystal structure of Beta2 bound to the Gs protein (PDB ID: 3SN6) did not give us a view of the direct interaction between the receptor and Beta subunit and Gamma subunit, and the abbreviated models can greatly reduce the molecular dynamics simulation time. After 5 ns's simulation, all the three models were equilibrated and the Root Mean Square Deviation (RMSD) plot of protein was shown in Figure 2A. 5 ns's simulation verified our previous speculation that even without the camel-nanobody, the Beta2 Adrenoceptor could still keep in active state. The simulation results were shown in Figure 2B. Due to the existence of G-alpha subunit of G protein, the TM5 and TM6 of receptors could not move inward spontaneously. In Figure 2B, the cyan ribbon was the Beta1 Adrenoceptor after simulation and the green ribbon was the C-terminal helix of G-alpha subunit of Gs protein after simulation. The yellow ribbon was an inactive crystal structure. Results showed that the distances between

active C-alpha of Gln254 in TM5 and Thr291in TM6, and inactive structures were 5.9 angstrom and 6.2 angstrom. The existence of C-terminal helix of G-alpha subunit of Gs protein obstructed the movement of these two transmembrane helixes.

Comparison of Beta Adrenoceptor-G Protein Interactions

To investigate the interactions of Beta Adrenoceptors and G proteins, we studied the complex models after molecular dynamics simulation. Results showed that they were in a canonical conformation revealed by the crystal structure of Beta2 adrenoceptor-Gs protein (PDB ID: 3SN6) but in some partial regions the residues generated different contacts, which affected the natural activity (Figure 2C).

Why Beta2 Adrenoceptor can activate Gi while Beta1 Adrenoceptor cannot? We therefore studied the extra residue contacts between Beta2 Adrenoceptor and Gi protein. After a careful research, it was found that Pro138 of Gi protein formed hydrophobic interactions with Ile344 of Beta2-AR and Lys232 of Gi protein generated an ionic interaction with Asp 337 of Beta2-AR. The RMSD plot of these two contacts was shown in Figure 3A. The figure showed that the contacts were stable during molecular dynamics simulation. However, the corresponding residues in Beta1 Adrenoceptor after amino acids alignment were Gln384 and Asn465 with which the former contacts could not be generated. This result suggested a possible reason why Gi is selectively activated by Beta2 Adrenoceptor.

It has also been found that the Beta1 adrenoceptor and Beta2 adrenoceptor had equal ability to activate Gs protein. We also studied the residues differences. To activate Gs, both of them showed nearly identical interactions. But there are two main differences (Figure 3B). The main differences were that Lys252 of Beta1-AR but Arg228 of Beta2-AR generated ionic interaction with Glu78, respectively.

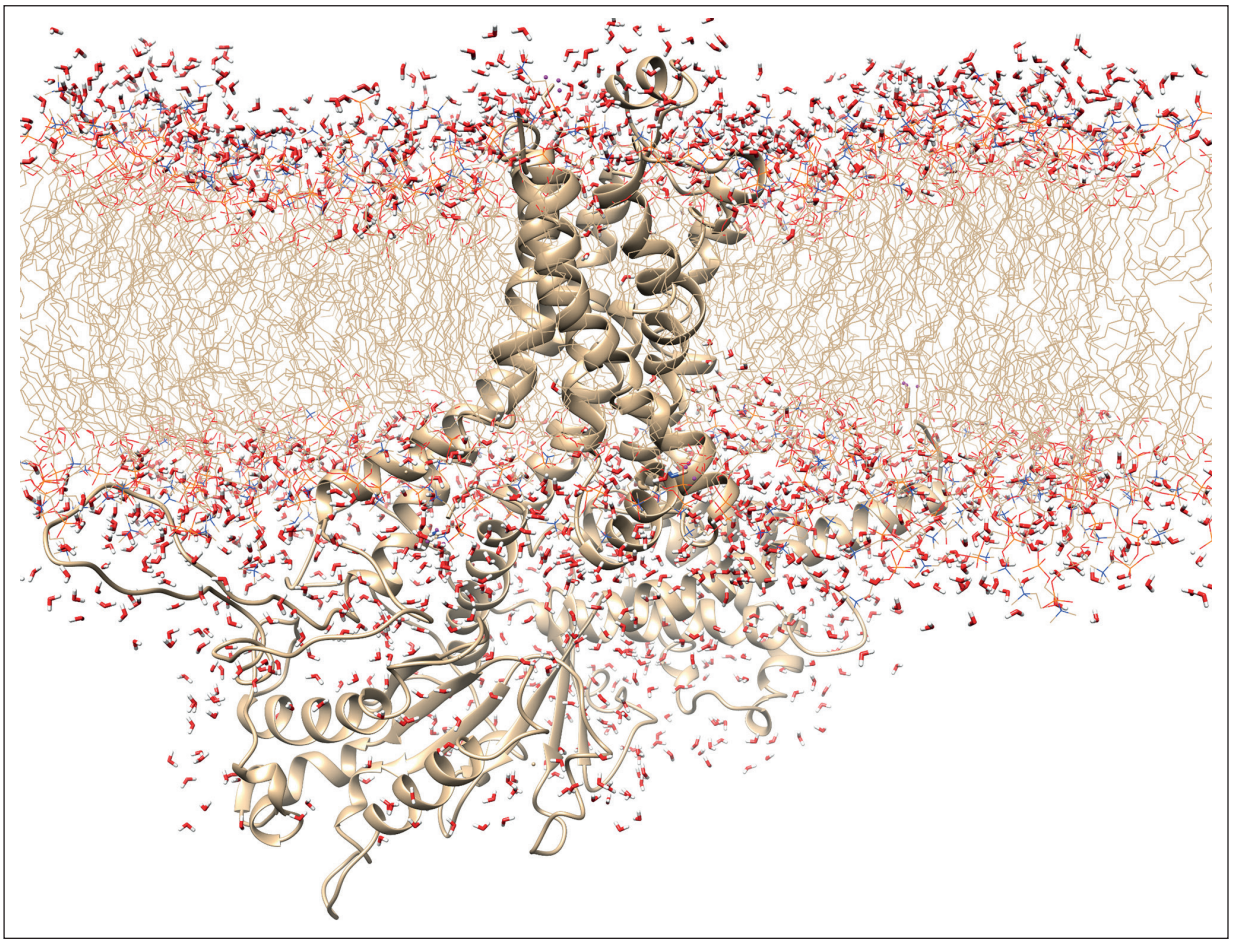

Figure 1. Orthostatic view of the simulated Beta2-Adrenoceptor and Galpha Subunit of Gs protein alpha subunit.

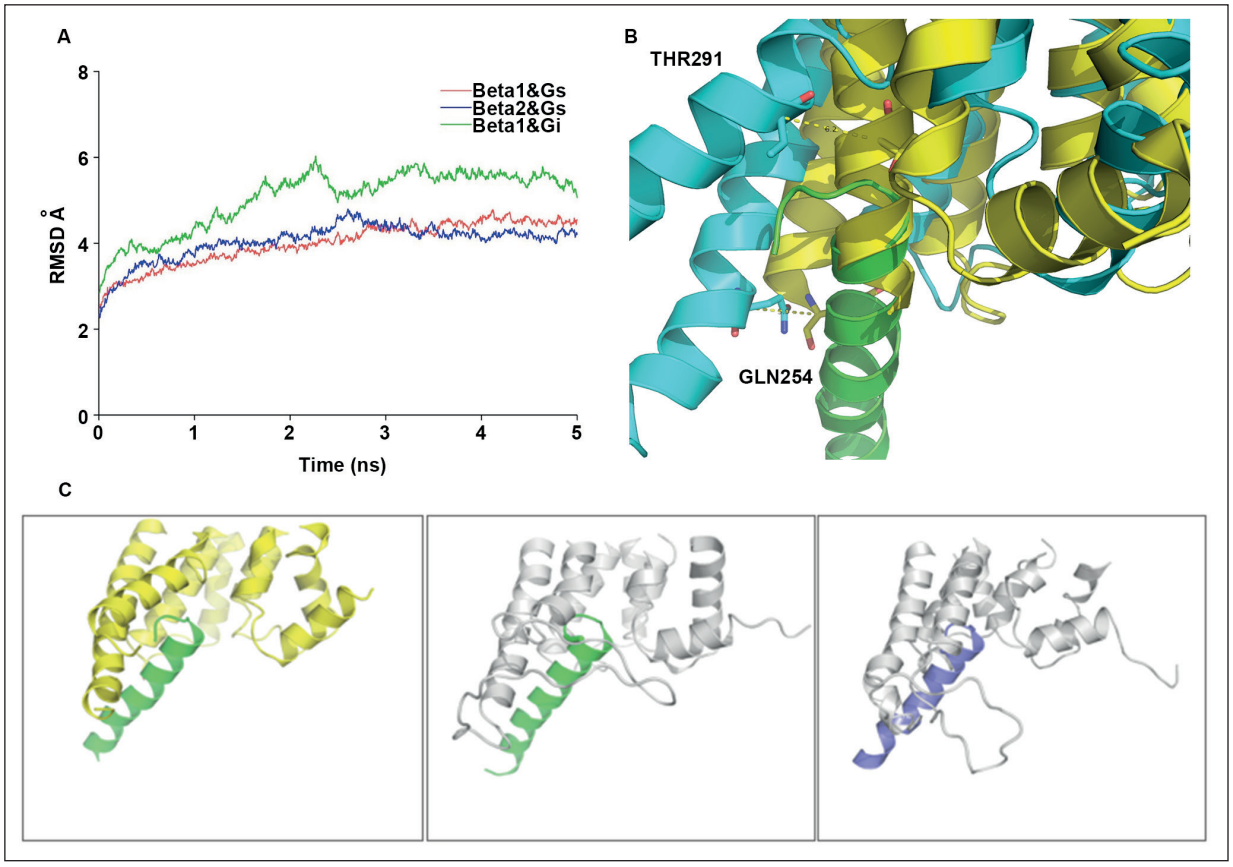

Figure 2. The three models were equilibrated after 5 ns. ***A***, RMSD plot of the three models. ***B***, Alignment of Beta2 adrenoceptor in inactive state (PDB ID: 2RH1) and models after molecular dynamic simulation. ***C***, Comparisons of receptor-G protein bindings. Yellow ribbon: Beta1 adrenoceptor; White ribbon: Beta2 adrenoceptor; Green ribbon: C-terminal helix of Gs protein alpha subunit; Cyan ribbon: C-terminal helix of Gi protein alpha subunit.

Arg165 of Beta1-AR but Lys140 of Beta2-AR generated ionic interaction with Asp133, respectively. These two contacts may neutralize the binding energy of these two proteins.

Structural Basis of Sodium Ion Binding

Liu et al[22] published a crystal structure of A2a adenosine receptor bound with a sodium ion which showed that the existence of sodium ion can block the entrance of agonist. They suggested that the agonist binding and the presence of sodium ion is exclusive. Herein, since the models we built were absent of agonist, we wanted to see whether the sodium can enter and extracellular binding pocket. Beyond our expectation, the sodium ion entered the binding pocket in just 5 ps and stabilized there, even when the molecular dynamics simulation finished (Figure 3C). This was the first modeling theoretical evidence that verified the sodium ion's importance in G-protein coupled receptors.

Conclusions

In this investigation, we performed molecular dynamics simulation of the complexes of Beta Adrenoceptors and G proteins. The results suggested that even without the camel-nanobody, the complex could still be stable to elucidate the interactions between GPCRs and g proteins, which told us that we could do the molecular dynamics simulation of a complex of a GPCR-G protein without nanobody bound. Then, molecular dynamics indicated the mechanism of protein-protein interaction, especially without the crystal structures bound together. Due to the fact that we may use homology modeling to generate complex of proteins, the simulation's time can be reduced greatly. Lastly, the molecular dynamics simulation here has revealed how this structure affects the activity, which will help us know or even predict further protein-protein interactions.

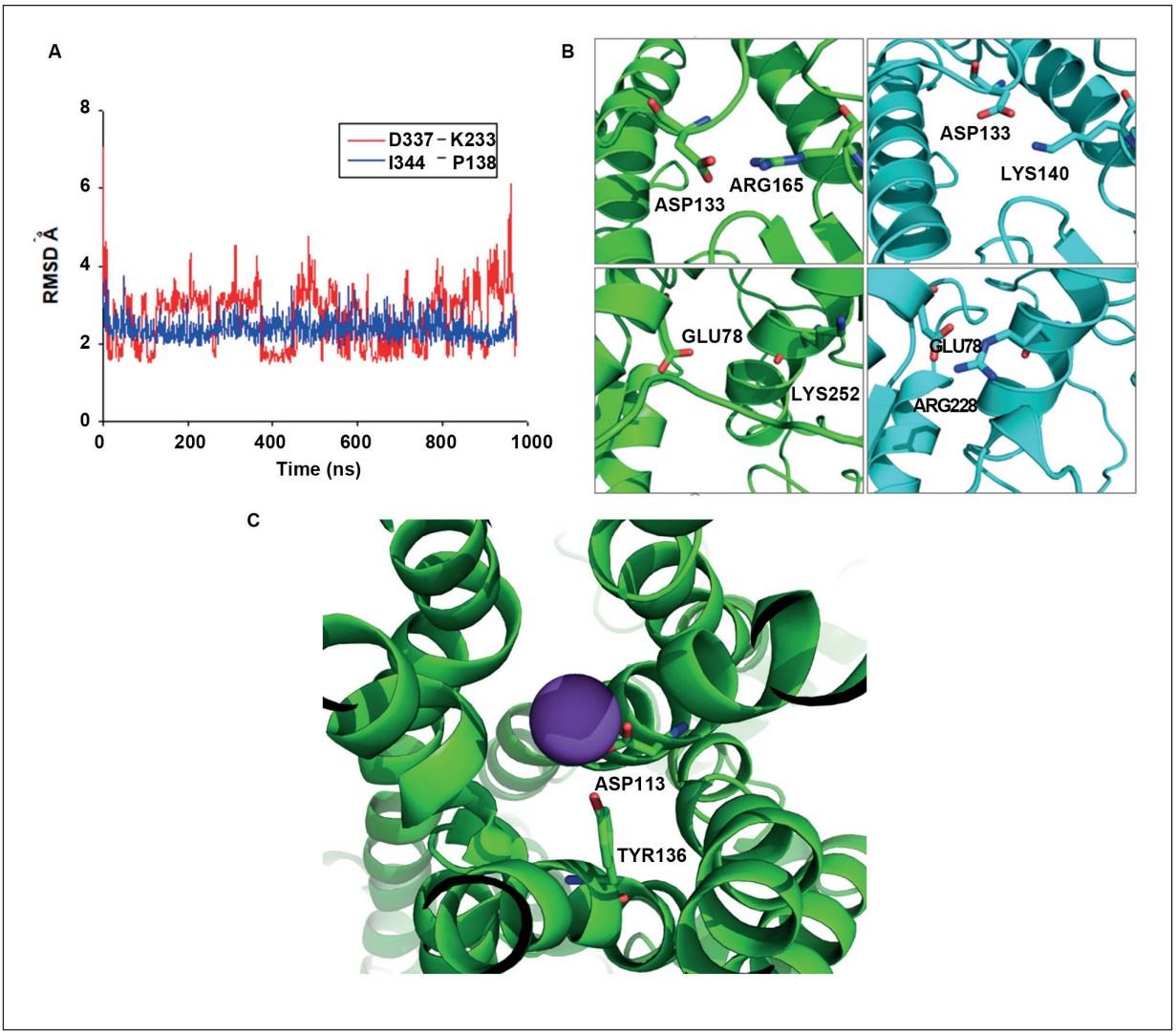

Figure 3. Different interactions of Beta-Adrenoceptor and G protein. ***A***, RMSD plot of distances of ionic interaction between D337 of Gi and K232 of Beta2-AR, and hydrophobic interaction between I344 of Gi and P138 of Beta2-AR. ***B***, Different interactions of Beta-Adrenoceptor and G protein. Green ribbon: Interactions of Beta1 Adrenoceptor and Gs protein. Cyan ribbon: Interactions of Beta2 Adrenoceptor and Gs protein. ***C***, Beta2-Gs complex. The sodium ion was shown in purple sphere.

Statement of Author Contributions

Z.-Y. LI (Zhenyu Li), C.-Y. SU (Chunying Su) and B. DING (Bo Ding) conceived and designed the experiments; Z.-Y. LI (Zhenyu Li) performed the experiments, analyzed the data and prepared the figures and manuscript. All the authors reviewed and approved the manuscript.

Acknowledgments

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Conflict of Interests

The Authors declared that they have no conflict of interests.

References

1) Lagerström MC, Schiöth HB. Structural diversity of G protein-coupled receptors and significance for drug discovery. Nat Rev Drug Discov 2008; 7: 339-357.
2) Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol 2003; 63: 1256-1272.
3) Stenkamp RE, Teller DC, Palczewski K. Crystal structure of rhodopsin: a G-protein-coupled receptor. Chembiochem 2002; 3: 963-967.
4) Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR,

Sanishvili R, Fischetti RF, Schertler GF, Weis WI, Kobilka BK. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. Nature 2007; 450: 383-387.

5) Hanson MA, Stevens RC. Discovery of new GPCR biology: one receptor structure at a time. Structure 2009; 17: 8-14.

6) Wang C, Wu H, Katritch V, Han GW, Huang XP, Liu W, Siu FY, Roth BL, Cherezov V, Stevens RC. Structure of the human smoothened receptor bound to an antitumour agent. Nature 2013; 497: 338-343.

7) Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weis WI, Sunahara RK, Kobilka BK. Crystal structure of the beta2 adrenergic receptor-Gs protein complex. Nature 2011; 477: 549-555.

8) Kahsai AW, Xiao K, Rajagopal S, Ahn S, Shukla AK, Sun J, Oas TG, Lefkowitz RJ. Multiple ligand-specific conformations of the beta2-adrenergic receptor. Nat Chem Biol 2011; 7: 692-700.

9) Chung KY, Rasmussen SG, Liu T, Li S, DeVree BT, Chae PS, Calinski D, Kobilka BK, Woods VL Jr, Sunahara RK. Conformational changes in the G protein Gs induced by the beta2 adrenergic receptor. Nature 2011; 477: 611-615.

10) Wacker D, Fenalti G, Brown MA, Katritch V, Abagyan R, Cherezov V, Stevens RC. Conserved binding mode of human beta2 adrenergic receptor inverse agonists and antagonist revealed by X-ray crystallography. J Am Chem Soc 2010; 132: 11443-11445.

11) Choe HW, Kim YJ, Park JH, Morizumi T, Pai EF, Krauss N, Hofmann KP, Scheerer P, Ernst OP. Crystal structure of metarhodopsin II. Nature 2011; 471: 651-655.

12) Standfuss J, Edwards PC, D'Antona A, Fransen M, Xie G, Oprian DD, Schertler GF. The structural basis of agonist-induced activation in constitutively active rhodopsin. Nature 2011; 471: 656-660.

13) Feng Z, Hou T, Li Y. Studies on the interactions between beta2 adrenergic receptor and Gs protein by molecular dynamics simulations. J Chem Inf Model 2012; 52: 1005-1014.

14) Dror RO, Arlow DH, Maragakis P, Mildorf TJ, Pan AC, Xu H, Borhani DW, Shaw DE. Activation mechanism of the beta2-adrenergic receptor. Proc Natl Acad Sci U S A 2011; 108: 18684-18689.

15) Webb B, Sali A. Comparative protein structure modeling using MODELLER. Curr Protoc Bioinformatics 2014; 47: 5.6.1-32.

16) Papadopoulos JS, Agarwala R. COBALT: constraint-based alignment tool for multiple protein sequences. Bioinformatics 2007; 23: 1073-1079.

17) Zhu M, Li M. Revisiting the homology modeling of G-protein coupled receptors: beta1-adrenoceptor as an example. Mol Biosyst 2012; 8: 1686-1693.

18) Deka H, Sarmah R, Sharma A, Biswas S. Modelling and characterization of glial fibrillary acidic protein. Bioinformation 2015; 11: 393-400.

19) Felts AK, Gallicchio E, Wallovist A, Levy RM. Distinguishing native conformations of proteins from decoys with an effective free energy estimator based on the OPLS all-atom force field and the Surface Generalized Born solvent model. Proteins 2002; 48: 404-422.

20) MacCallum JL, Tieleman DP. Calculation of the water-cyclohexane transfer free energies of neutral amino acid side-chain analogs using the OPLS all-atom force field. J Comput Chem 2003; 24: 1930-1935.

21) Li YJ, Zhu HX, Zhang D, Li HC, Ma P, Huang LY. Novel endogenous negative modulators of platelet function as potential anti-thrombotic targets. Eur Rev Med Pharmacol Sci 2017; 21: 3146-3158.

22) Liu W, Chun E, Thompson AA, Chubukov P, Xu F, Katritch V, Han GW, Roth CB, Heitman LH, IJzerman AP, Cherezov V, Stevens RC. Structural basis for allosteric regulation of GPCRs by sodium ions. Science 2012; 337: 232-236.