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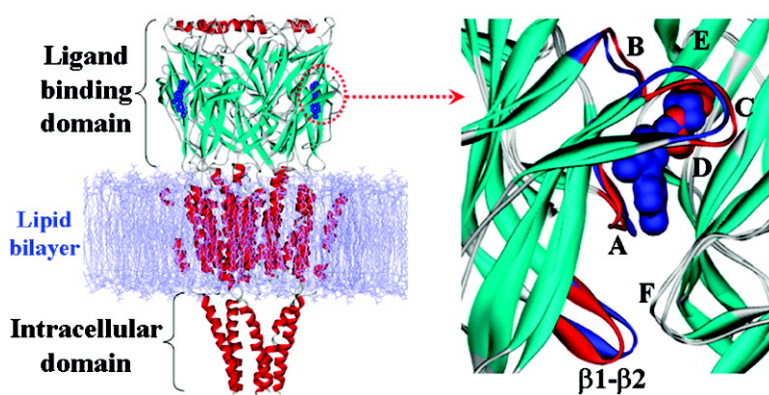
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Modeling Differential Binding of α4β2 Nicotinic Acetylcholine Receptor with Agonists and Antagonists

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Abstract: Three-dimensional structures of both the open- and closed-channel states of α4β2 receptor have been modeled and used to study their binding with representative agonists and antagonists. The obtained binding structures and free energies consistently reveal that antagonists bind more favorably with the closed-channel state and agonists bind more favorably with the open-channel state. The computational insights have led us to propose a computational strategy and protocol predicting whether a receptor ligand is an agonist or antagonist. Using the computational protocol, one only needs to calculate the relative binding free energies for a ligand binding with the open- and closed-channel structures. The ligand is predicted to be an agonist if the binding free energy calculated for the ligand binding with the open-channel state is significantly lower than that for its binding with the closed-channel state. If the binding free energy of a ligand with the open-channel state is higher than that with the closed-channel, the ligand is predicted to be an antagonist. The binding free energies calculated for all of the ligands binding with their most favorable channel states of the receptor are all close to the corresponding experimentally derived binding free energies. The new computational insights obtained and novel computational strategy and protocol proposed in this study are expected to be valuable in structure-based rational design of novel agonists/antagonists of nAChRs as therapeutic agents.

Introduction

Nicotinic acetylcholine receptors (nAChR) are a family of well-studied ligand-gated ion channels prevailing in central nervous system (CNS).[1–7] These receptors directly mediate fast signal transmission at chemical synapse by binding with neurotransmitter molecules. Abnormal opening—closing of these channels contributes to neurodegenerative disorders, resulting in several kinds of severe diseases, including Alzheimer's diseases, Parkinson's diseases, dyskinesias, Tourette's syndrome, schizophrenia, attention deficit disorder, anxiety, and pain, as well as nicotine addiction.[2] Among a series of the pentameric subtypes of these receptors, α4β2 subtype (stochastically as (α4)2(β2)3, i.e., α4β2α4β2β2) has emerged as the most attractive therapeutic target for the treatment of these diseases because α4β2 nAChR accounts for a large number of the high-affinity nicotine binding sites in the brain.[8] A typical agonist is a molecule that can bind with a nAChR receptor, bringing about conformational change of the receptor and finally leading to

the opening of the channel for the permeation of Na⁺ ion. When an antagonist is bound, the conformational change of the nAChR receptor is not sufficient for the channel opening and, therefore, no Na⁺ permeation.[3,4] Activation of α4-containing receptors is sufficient for nicotine-induced reward, tolerance, and sensitization effects. The β2-containing receptors are necessary for the maintenance of nicotine self-administration, and the blockade of the high-affinity α4- and β2-containing nAChRs by a competitive antagonist attenuates the rewarding effects of nicotine.[1,9,10] During the past decades, a large number of nAChR ligands, especially agonists, were discovered.[11–18] Among these ligands, epibatidine and its analogs were typical α4β2 nAChR agonists. Meanwhile, α4β2 nAChR antagonists have become

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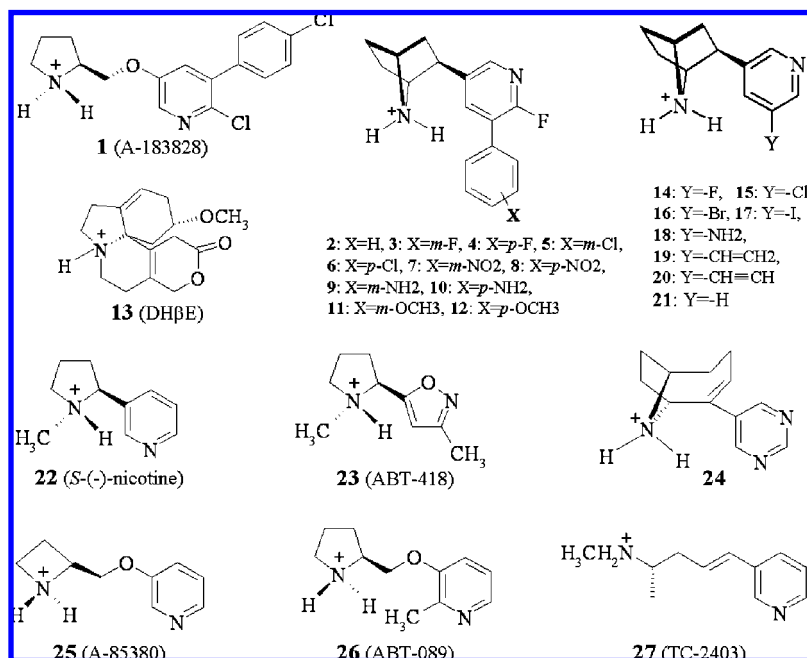
Figure 1. Molecular structures of representative ligands for α4β2 nAChR.

more attractive, particularly after a few types of novel competitive antagonists have been developed recently (Figure 1).[19−23] In order to design more potent and diverse antagonistic therapeutics, it is particularly important to understand how α4β2 nAChR can be functionally antagonized.

Recent studies[3−5,24−31] on pharmacological and structural characteristics of nAChRs were performed on the extracellular ligand-binding domain (LBD) of nAChRs using the homologous acetylcholine binding protein (AChBP) as the prototype. Ligand binding was explored by AChBP in complex with different agonists and antagonists such as nicotine, epibatidine, and methyllycaconitine (MLA).[24−26] The refined 4 Å resolution electron microscopy (EM) structure of the heteropentameric muscle-type (α)2(βδγ) receptor led to a better understanding of the structure and ligand-recognition of nAChRs, although the ligand-binding site in this structure was obviously distorted by inter- and intrasubunit interactions.[27,28] Other functional studies were focused on the molecular pathway for the coupling

of agonist binding to the channel gating.[5,29−31] Based on these studies, possible molecular mechanisms[30,31] of agonist binding and channel opening were proposed and the proposed mechanisms were thought applicable to most nAChR subtypes. According to the suggested channel-opening mechanism, a competitive antagonist must be able to potently bind with the closed-channel state of the receptor and prevent the conformational changes necessary for the channel opening. Because hyperactive opening-closing of α4β2 nAChR can produce nicotine addiction,[1,2] antagonizing α4β2 nAChR has become a clearly promising therapeutic strategy.

Current efforts on rational design and discovery of α4β2 nAChR antagonists are mainly based on the empirical structure−activity relationship (SAR) analysis of certain derivatives of some known agonists. Such rational design is usually limited by the inadequate structural diversity of the designed ligands.[20,23] For this purpose, we modeled the LBD structure of α4β2 receptor (in the open-channel state) and developed a new computational strategy and scoring function to predict the binding affinities of various agonists binding with nAChRs.[32,33] The calculated binding free energies are all in good agreement with available experimental data. However, it is especially challenging for molecular modeling and molecular dynamics (MD) simulation to predict whether a given ligand should be an agonist or antagonist before the ligand is actually synthesized and used for a practical functional assay (e.g., electrophysiological measurement) with the receptor. So, currently, there is no computational approach available for predicting the agonism or antagonism of a nAChR ligand. It is interesting to develop a reliable and efficient computational approach which can be used to predict whether a nAChR ligand should be an agonist or antagonist for the purpose of agonist or antagonist design.

Theoretically, to computationally predict whether a nAChR ligand should be an agonist or antagonist, one may first

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determine how the ligand binds with the closed-channel state (the rest state) of the nAChR and then carry out a sufficiently long MD simulation on the determined nAChR-ligand binding structure in a reasonable physiological environment. If the ligand is an agonist, then the channel should eventually open during the MD simulation. If the ligand is an antagonist, then the channel should not open during the MD simulation. Practically, this theoretically "reasonable" approach does not work for a nAChR. This is because the average time required to open nAChR channels is in milliseconds (ms), e.g., ~59 ms for α4β2 nAChR[10,29] and, therefore, the MD simulation on a nAChR-ligand complex must be performed for at least many milliseconds to be really meaningful. Such a time scale is insurmountable for a fully relaxed (real-time) MD simulation (with a usual time step of 1 or 2 fs) of a protein system as large as a nAChR on any supercomputer in the World at this point of the time. Currently, a meaningful MD simulation (with a usual time step of 1 or 2 fs) on a fully solvated nAChR system can only be performed for nanoseconds by using supercomputing time in days.

In fact, targeted MD simulations (i.e., the MD simulations with certain artificial forces that accelerate the change of nAChR structure from the starting closed/open-channel state to the targeted open/closed-channel state) have been performed on nAChRs to study some major molecular motions related to the opening and closing of nAChR channels.[5] As expected, the artificial forces speeded up the opening/closing of the channel so that the channel opening/closing could be simulated in only nanoseconds. However, when the targeted MD simulation is performed on a nAChR—ligand complex, the channel opening/closing can always be observed due to the use of the artificial forces no matter whether the ligand is an agonist or antagonist. So, the targeted MD simulations cannot be used to predict whether a nAChR ligand is agonist or antagonist.

In the present study, we aimed to develop a practical and fast computational approach which can be used to predict whether a nAChR ligand is an agonist or antagonist. For this purpose, starting from the agonist-binding state of the LBD structure of α4β2 nAChR modeled in our previous studies,[32,33] we first constructed both the closed- and open-channel models of the α4β2 receptor structure using available structures of AChBP and (α)2(βδγ) receptor[24,25,28,29] as templates. We then modeled the binding structures and performed binding free energy calculations for a set of 27 representative ligands including (−)-deschloroepibatidine analogs (Figure 1), some of which were experimentally demonstrated as α4β2 nAChR antagonists (see Table S1 of Supporting Information). The computational results indicate that, for all of the ligands examined, antagonists bind more favorably with the closed-channel state, whereas agonists[12–18] bind more favorably with the open-channel state of the α4β2 receptor. The binding free energies calculated for antagonists binding with the closed-channel state and for agonists binding with the open-channel state are all in good agreement with the experimentally measured binding affinities, suggesting that the calculated relative binding free energies may be used to predict whether a ligand of the receptor is an agonist or antagonist.

Computational Methods

The 2.45 Å resolution X-ray structure of AChBP in antagonist methyllycaconitine (MLA)-bound conformation (Protein Data Bank ID 2BYR)[25] was used as the template to model the LBD (i.e., the extracellular domain) of the closed-channel α4β2 receptor, and the transmembrane (TM) domain of the 4 Å resolution electron microscopy (EM) structure of (α)2(βδγ) receptor (Protein Data Bank ID 2BG9)[28] was used to model the remaining part of each subunit of the closed-channel α4β2 receptor (i.e., the TM domain (TMD) containing transmembrane helices M1, M2, M3, and M4 of each subunit, and the intracellular domain composed of all 5 subunits, also see Figure 2S in Supporting Information). In light of recent structural understanding of the gating mechanism,[27,28,30,31] the open-channel α4β2 receptor was modeled in a similar way, but using a different template for the LBD and making some minor structural changes on the TMD. The LBD model of open-channel α4β2 receptor was taken from our previously modeled LBD structure with agonist (−)-deschloroepibatidine bound[32] which was modeled by using the 2.2 Å resolution X-ray crystal structure of nicotine-bound AChBP structure as the template (Protein Data Bank ID 1UW6).[24] The aforementioned TMD template was used to model the structure of the TMD, but the αPro269 in α4β2 nAChR was changed to the *cis*-conformation and, accordingly, the M2 helices were self-rotated about 12° in order to keep the gating ring (e.g., αLeu248 and βLeu251) away from the central pore of the channel. M1, M3, and M4 helices in the initial model of the open-channel receptor were kept rigid, while the M2 helices were rotated, according to the experimental insights.[27,28,30,31] The initial closed- and open-channel α4β2 models were inserted into a pre-equilibrated phospholipid bilayer structure. The structural models were refined carefully by performing further molecular modeling including a series of energy minimization processes.

Docking of the 20 antagonists and 7 agonists (Figure 1) into the binding site at the interface of the α4 and β2 subunits of α4β2 receptor in both the closed- and open-channel states was performed by AutoDock 3.0.5 program[34] in a similar way as we did in our previous studies.[32,33] For each ligand, the favorable binding structure was selected to be the one which has both the lowest binding energy and the reasonable orientation in the binding site. The selected favorable binding structure was then used as an initial complex structure subjected to energy-minimization by using the Sander module of the Amber8 program.[35] The binding free energy for each of the final energy-minimized receptor—ligand complex was calculated using the scoring function developed in our previous study[33] in the functional form as:

$$\Delta G_{bind} = \alpha(\Delta G_{AD} - E_0) + \Delta G_{HB} + \Delta G_{LRE} \qquad (1)$$

in which ΔG_{HB} is the hydrogen bonding energy calculated by using a revised equation (i.e., eq 2 in Supporting Information), ΔG_{AD} is the binding free energy calculated by using standard scoring function in the AutoDock 3.0.5 program,[34] E_0 is a universal empirical parameter, and ΔG_{LRE} is the additional long-range electrostatic interaction energy.[33]

The detailed molecular modeling and computational procedure is provided in Supporting Information.

Results and Discussion

Binding with Closed-Channel α4β2 Receptor. We only discuss the binding structures for the protonated molecular species of ligands, as the binding with the deprotonated structures is always negligible compared to the corresponding binding with the protonated species (see below for the energetic results). Figure 2 depicts the structural model of closed-channel α4β2 receptor, using its binding with an antagonist (compound **1**) as an example. For comparison, the open-channel structure binding with agonists, represented by compound **21**, is also superimposed (blue in Figure 2). In comparison of the closed-channel structure with its open-channel structure, a significant

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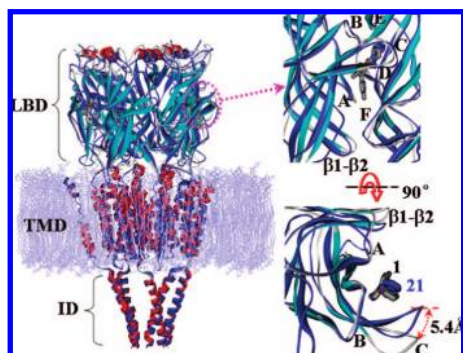
Figure 2. Representation of the energy-minimized closed-channel α4β2 nAChR structure binding with antagonist **1**. Left panel is a side view of the whole receptor structure situated in the phospholipid bilayer and represented by its secondary structure. LBD refers to ligand binding domain, TMD to transmembrane domain, and ID to intracellular domain. The antagonist **1** is shown in stick mode. For comparison, the open-channel structure is superimposed and in blue color, whereas the phospholipid bilayer is in light blue color. The right upper panel is the local view of one of the two ligand-binding sites at the extracellular ligand-binding domain of the receptor, in which antagonist **1** is shown in stick and the agonist **21** is shown in stick and blue color. The right down panel is a top view. Antagonist **1** is shown in stick and agonist **21** is in blue color. For clarity, the β2 subunit and the hydrogen atoms for ligands 1 and 21 are not shown in the figure. Labeled are the functional loops in the right panels (A-F, and β1-β2 loop). In comparison of the closed-channel structure with the open-channel structure, one of the most obvious differences exists in the motion of the C loop induced by the ligand binding. Such a difference is represented by the red-curved arrow and labeled with the distance.

difference exists in the shape and size of the ligand-binding site at the interface of α4 subunit and β2 subunit. The ligand-binding site in the closed-channel structure is more bulky and more extended along the interface of the two subunits. As shown in Figure 2, the binding site of antagonist **1** in the closed-channel structure is extended down toward the membrane along the subunit interface, whereas the binding site of agonist **21** (blue in Figure 2) in the open-channel state is much more compact. The C-loop moved in ∼5.4 Å toward the bound agonist **21** in the open-channel receptor, compared to the C-loop in the closed-channel receptor bound with antagonist **1**. Because of the binding of agonist **21**, the inner sheets of the α4 subunit in the open-channel receptor take more clock-wise rotation than that in the closed-channel receptor binding with antagonist **1**, resulting in the B and C loops wrapping much closer to the bound agonist **21** (blue in the right panel of Figure 2).

The modeled binding structures of the closed-channel receptor binding with other ligands are provided and discussed in Supporting Information. In general, all of the antagonists (**1**–**20**) can favorably bind with the closed-channel α4β2 receptor, whereas all of the agonists (**21**–**27**) cannot form favorable binding structures with closed-channel α4β2 receptor. The binding structures modeled for the agonists are generally rather loose, due to the relatively smaller molecular size compared to the antagonists. In particular, compared to the strong N–H···O hydrogen bonding between closed-channel α4β2 receptor and antagonists **1**–**20**, the corresponding N–H···O hydrogen bonds between closed-channel α4β2 receptor and agonists **21**–**27** are much weaker, with the H···O distances being generally longer than 2.0 Å.

Binding with open-channel α4β2 receptor. The binding pocket in the extracellular domain of the open-channel receptor is significantly smaller than that of the closed-channel receptor. For this reason, antagonists **1** to **20** that fit the binding pocket of the closed-channel α4β2 receptor very well do not fit the

binding pocket of the open-channel α4β2 receptor well. Antagonists **1**–**12** and **14**–**20** even cannot possess an orientation similar to those in the corresponding closed-channel α4β2 receptor structures discussed above. For example, the cationic head of antagonist **1** is partially embedded into the binding pocket and no longer can form the aforementioned N–H···O hydrogen bond with αTrp149 of open-channel α4β2 receptor (see Figure S5 in Supporting Information). The chlorine-substituted phenyl group on the tail of antagonist **1** is exposed to the solvent at the interface of open-channel α4β2 receptor. The binding site of open-channel α4β2 receptor even cannot accommodate an antagonist as large as **13** at all.

Hence, a ligand with a large molecular size cannot fit the binding site of the open-channel α4β2 receptor. However, it does not mean that any molecule with a small size can fit the binding site of the open-channel α4β2 receptor. Whether a ligand can fit the binding site or not is dependent on its specific molecular shape and specific intermolecular interactions with the receptor. The binding of open-channel α4β2 receptor with agonists **21**–**27** have been modeled in our previous studies[32,33] using a LBD model of α4β2 receptor. The important specific intermolecular interactions identified in our previous studies[32,33] include various hydrogen bonds and cation-π interactions between the receptor and agonists. In the present study, we further modeled the binding of these agonists with a more complete structural model of open-channel α4β2 receptor. The receptor-agonist binding modes modeled in this study are essentially the same as the corresponding receptor-agonist binding modes reported in our previous studies.[32,33] Each of these agonists fits well with the binding site of open-channel α4β2 receptor.

Binding Free Energies. The modeled receptor−ligand binding structures were used to evaluate the corresponding binding free energies by using our recently developed and validated novel scoring function (without recalibrating any parameter in the function),[33] i.e., eq 1. We note that only LBD of the receptor was considered in our previous calculations[33] on ΔG_{LRE} (the long-range electrostatic interactions) in eq 1. In the present study, we also tested using the entire structural models of the receptor, instead of the LBD only, and found that the total contribution of the non-LBD part of the receptor to ΔG_{LRE} is negligible for all of the modeled binding structures. This is because the non-LBD part of the receptor is far away from the ligand-binding site. Hence, we elected to ignore the non-LBD part of the receptor in the final ΔG_{LRE} calculations in order to be completely consistent with our previous computational study[33] on the agonists binding with the receptors. According to the calculated energetic results, for each ligand binding with the receptor (open- or closed-channel state), the deprotonated species always has a much lower binding affinity (with the calculated ΔG_{bind} value being higher by more than 4.5 kcal/mol) than that of the corresponding protonated species with the same receptor state (see Table S2 in Supporting Information). So, the dominant molecular species of the ligand binding with the receptor is always the protonated species for all of the ligands binding with both the open- or closed-channel states of the receptor; the binding with the deprotonated species is always negligible.

Summarized in Table 1 are the calculated binding free energies for all ligands (**1**–**27**) binding with both the open- and closed-channel states of α4β2 receptor in comparison with the corresponding binding free energies derived from the experimental dissociation constant (K_d) values (based on the well-known thermodynamic equation $\Delta G_{bind} = -RT \log K_d$ where T

Table 1. Calculated Binding Free Energies (ΔG_{bind}^{calc} in kcal/mol at T = 298.15 K) for Ligands **1**−**27** Binding with the Open- and Closed-Channel States of α4β2 Receptor in Comparison with the Experimentally-Derived Binding Free Energies (ΔG_{bind}^{expt})[a]

no.[b]	K_d (expt, in nM)	ref	ΔG_{bind}^{calc} (kcal/mol)		ΔG_{bind}^{expt} (kcal/mol)
			closed	open	
1	0.461	[19]	−11.3	−8.2	−12.7
2	0.24	[20,21]	−11.3	−7.2	−13.1
3	0.087	[20,21]	−12.5	−7.5	−13.7
4	0.029	[20,21]	−12.7	−7.6	−14.4
5	0.073	[20,21]	−12.1	−7.9	−13.8
6	0.044	[20,21]	−12.6	−7.7	−14.1
7	0.053	[20,21]	−12.4	−7.7	−14.0
8	0.009	[20,21]	−13.2	−7.9	−15.1
9	0.16	[20,21]	−11.9	−7.4	−13.4
10	0.095	[20,21]	−12.2	−7.6	−13.7
11	0.12	[20,21]	−11.9	−8.1	−13.5
12	0.06	[20,21]	−12.5	−8.2	−13.9
13	35.0	[22]	−10.3	n/a[c]	−10.2
14	0.037	[23]	−12.7	−9.9	−14.2
15	0.055	[23]	−12.9	−9.5	−14.0
16	0.050	[23]	−12.7	−10.1	−14.1
17	0.059	[23]	−12.6	−9.8	−14.0
18	0.097	[23]	−11.9	−9.8	−13.7
19	0.260	[23]	−13.0	−10.0	−13.1
20	0.020	[23]	−13.1	−9.5	−14.6
21	0.020	[13]	−7.9	−13.1	−14.6
22	1.10	[12]	−7.3	−10.4	−12.2
23	52.9	[14]	−7.3	−10.0	−9.9
24	2.45	[15]	−7.7	−11.6	−11.7
25	0.015	[16]	−6.5	−13.1	−14.8
26	39	[17]	−7.3	−10.9	−10.1
27	26.0	[18]	−6.3	−10.9	−10.3

[a] The ΔG_{bind}^{expt} values are derived from the measured K_d values according to the well-known thermodynamic equation: $\Delta G_{bind}^{expt} = -RT \log K_d$ at T = 298.15 K. [b] Listed in Supporting Information (Table S1) are the calculated values for the three components of ΔG_{bind}, i.e., ΔG_{AD}, ΔG_{HB}, and ΔG_{LRE} for all these ligands with both closed-channel and the open-channel α4β2 receptor. Names of some ligands are: **21**, (−)-deschloroepibatidine; **22**, *S*-(−)-nicotine; **23**, ABT-418; **24**, (5-pyridyl)-9-azabicyclo[4.2.1]non-2-ene; **25**, A-85380; **26**, ABT-089; **27**, TC-2403. [c] Not applicable, because ligand **13** does not fit the binding pocket at all and, thus, cannot bind with the open-channel α4β2 receptor.

= 298.15 K). The more detailed energetic values for the three components of ΔG_{bind}, i.e., ΔG_{AD}, ΔG_{HB}, and ΔG_{LRE}, are provided in Supporting Information. As seen in Table 1, the ΔG_{bind} value calculated for each of the antagonists (**1**−**20**) binding with the closed-channel α4β2 receptor is much lower than that calculated for its binding with the open-channel receptor. In contrast, the ΔG_{bind} value calculated for each of the agonists (**21**−**27**) binding with the open-channel α4β2 receptor is much lower than that calculated for its binding with the closed-channel receptor. The calculated relative binding free energies are qualitatively consistent with the aforementioned notion concerning the modeled binding structures, i.e., all of the antagonists bind more favorably with the closed-channel receptor, whereas all of the agonists bind more favorably with the open-channel receptor. The difference between the ΔG_{bind} values calculated for a ligand binding with the open- and closed-channel structures is mainly attributed to the difference between the calculated ΔG_{HB} values (see Supporting Information).

The data in Table 1 indicate that the binding free energies calculated for the favorable binding of antagonists (**1**−**20**) with the closed-channel receptor and for the favorable binding of agonists (**21**−**27**) with the open-channel receptor are all close to the corresponding experimentally derived binding free energies. The good agreement between the computational and

experimental data suggests that the determined binding structures and calculated binding free energies are reasonable.

To have a better energetic understanding of the channel opening following agonist binding, it is important to know the energy difference between the open-channel and closed-channel states. Unfortunately, a reliable computational protocol to predict the energy difference between the two states has not developed yet. Nevertheless, some rough energetic information may be drawn from an analysis of our calculated relative binding free energies in comparison with available experimental data. Recent experimental and computational studies[5,7,30] have revealed that the transition between the closed-channel (**C**) and the open-channel (**O**) of nAChRs is very energy-efficient, and that the conformational change during the transition is spontaneous, e.g., the closed-channel can make excursions into the open-channel by dynamic conformational change.[7] Based on the single channel kinetic analysis on human muscle type nAChR,[30] the opening and closing rate constants were determined to be 44,000 s^{-1} (k_O) and 1700 s^{-1} (k_C), respectively. According to these experimental data, the energy difference ($\Delta G_{OC} = -RT \ln k_O/k_C$) between the open- and closed-channel states is estimated to be ∼1.9 kcal/mol at T = 298.18 K. So, the total energy of the closed-channel state is ∼1.9 kcal/mol higher than that of the open-channel with an agonist in the binding site. Assuming that all nAChRs have the similar energy difference between the open- and closed-channel states, such a small energy difference suggests that the opening of a nAChR channel can be easily modulated by agonist binding. An appropriately combined use of these interesting experimental data[5,7,30] and our calculated relative binding free energies leads us to better understand why an agonist can open the channel (to activate the nAChR receptor), whereas an antagonist cannot. As seen in Table 1, the binding free energies calculated for the agonists binding with the open-channel are lower than the corresponding data with the closed-channel by ∼4.2 kcal/mol in average. Based on this average difference in binding free energy and the aforementioned energy difference of ∼1.9 kcal/mol in the total energy of the receptor with an agonist in the binding site, the total energy of the closed-channel state is estimated to be ∼2.3 kcal/mol lower than that of the open-channel when there is no agonist in the binding site. Based on this roughly estimated difference in total energy of the free receptor, the energetic requirement for an agonist is that its binding free energy with the open-channel state should be lower than its binding free energy with the closed-channel state by at least ∼2.3 kcal/mol.

We must point out that the above energy estimation is by no means accurate, because of the computational errors in binding free energy calculations and the possibility that the energy difference for different receptor subtypes may be significantly different. Nevertheless, the above analysis of the computational and experimental data at least can suggest that the difference between the open-channel and closed-channel states of the free receptor in total energy must be as small as a few kcal/mol, as it is estimated to be ∼2 kcal/mol in the present study. When the binding free energy of a ligand with the open-channel is close to or higher than that with the closed-channel, the ligand will behave like an antagonist. It should be pointed out that the relative binding free energies are only a necessary condition for the channel opening which may be the result of the C-loop movement and turn of the α4 subunit.[30,31] Within the 20 antagonists listed in Table 1, the calculated binding free energies with the open-channel state are higher than the corresponding

data with the closed-channel by ~2.1 to ~5.3 kcal/mol, which explains why these 20 ligands are antagonists.

Before a ligand (agonist or antagonist) binds with the receptor, the channel is in the close-channel state. After a ligand binds with the receptor, whether the channel will open or not is dependent on whether the ligand is an agonist or antagonist. When the ligand is an agonist, the channel tends to open because the agonist binding with the open-channel state is stronger or the complex of the agonist with the open-channel receptor is more stable than that with the closed-channel receptor. When the ligand is an antagonist, the channel will not open because the antagonist binding with the closed-channel state is stronger or the complex of the antagonist with the closed-channel receptor is more stable than that with the open-channel receptor.

Further, the above data and analysis clearly point to a new computational protocol for predicting whether a receptor ligand should be an agonist or antagonist. Specifically, to predict whether a ligand should be an agonist or antagonist, one can first computationally model the binding of this ligand with both the open- and closed-channel states of the receptor and calculate the corresponding binding free energies by using eq 1. Then the calculated relative binding free energies can be used to predict whether the ligand should be an agonist or antagonist. The ligand is predicted to be an agonist if the binding free energy calculated for the ligand binding with the open-channel receptor is significantly lower than that for the ligand binding with the closed-channel receptor; otherwise, the ligand is predicted to be an antagonist. The computational protocol is expected to be applicable to other subtypes of nAChRs, as all subtypes of nAChRs, e.g., $\alpha 3\beta 4$ and $\alpha 7$, share the similar structural and pharmacological features.[4,5,8] In addition, the general computational strategy may also be used to differentiate antagonists from agonists of other ligand-gated ion channels in the Cys-loop superfamily, such as γ-aminobutyric acid receptor A (GABA$_A$), as these channel proteins share the similar molecular mechanism of channel opening and closing.[2−4,30,31]

Conclusion

The present computational modeling shows that the structure of the closed-channel $\alpha 4\beta 2$ receptor differs from that of the open-channel receptor mainly in the shape and size of the ligand-binding site. The difference is related to the local motions of the B-loop and C-loop of the LBD, as well as the lining of M2 helix triggered by the conformational changes of the LBD.

The modeled receptor−ligand binding structures and calculated binding free energies consistently reveal that all of the antagonists bind more favorably with the closed-channel state of the receptor, whereas all of the agonists bind more favorably with the open-channel state. These results help to better understand why an agonist can open the channel, whereas an antagonist cannot. The binding free energies calculated for the favorable binding of antagonists with the closed-channel state and for the favorable binding of agonists with the open-channel state are all close to the corresponding experimentally derived binding free energies. The good agreement between the computational and experimental data suggests that the determined binding structures and calculated binding free energies are reasonable.

All of the computational results have led us to propose a novel computational strategy and protocol that can be used to theoretically predict whether a nAChR ligand should be an agonist or antagonist. According to the computational protocol, one only needs to calculate the relative binding free energies for a ligand binding with both the open- and closed-channel states of the receptor and, thus, determine the most favorable channel state of the receptor binding the ligand, as the agonist and antagonist bind more favorably with the open- and closed-channel states, respectively. This protocol and the general computational strategy are expected to be valuable in structure-based rational design of novel agonists and antagonists of nAChRs as therapeutic agents. For example, a possibly more potent agonist of $\alpha 4\beta 2$ receptor may be designed to have a more favorable binding with the open-channel structure, whereas a possibly more potent antagonist of $\alpha 4\beta 2$ receptor may be designed to have a more favorable binding with the closed-channel receptor.

Supporting Information Available: Computational details, more structural and energetic information including two tables and six figures, and complete citations of refs 12, 14, 16, 19, and 35. This material is available free of charge via the Internet at http://pubs.acs.org.

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