Recognition of the ligand-induced spatiotemporal residue pair pattern of β2-adrenergic receptors using 3-D residual networks trained by the time series of protein distance maps

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ABSTRACT

G protein-coupled receptors (GPCRs) are promising drug targets because they play a large role in physiological processes by modulating diverse signaling pathways in the human body. The GPCR-mediated signaling pathways are regulated by four types of ligands—agonists, neutral antagonists, partial agonists, and inverse agonists. Once each type of ligand is bound to the binding site, it activates, deactivates, or does not perturb signaling by shifting the conformational ensemble of GPCRs. Predicting the ligand’s effect on the conformation at the binding moment could be a powerful screening tool for rational GPCR drug design. Here, we detected conformational differences by capturing the spatiotemporal residue pair pattern of the ligand-bound β2-adrenergic receptor (β2AR) using a 3-dimensional residual network, 3D-ResNets. The network was trained with the time series of protein distance maps extracted from hundreds of molecular dynamics (MD) simulation trajectories of ten β2AR-ligand complexes. The MD system was constructed with a lipid bilayer embedded in an inactive β2AR X-ray crystal structure and solvated with explicit water molecules. To train the network, three hyperparameters were tested, and it was found that the number of MD trajectories in the training set significantly affected the model’s accuracy. The classification of agonists and neutral antagonists was successful, but inverse agonists were not. Between the agonists and antagonists, different residue pair patterns were spotted on the extracellular loop segment. This result demonstrates the potential application of a 3-D neural network in GPCR drug screening, as well as an analysis tool for protein functional dynamics.

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1. Introduction

G-protein coupled receptors (GPCRs) belong to the largest family of membrane proteins and are one of the critical pharmacological targets. Approximately 35% of approved drugs target them in various diseases because of their ubiquitous nature.[1] Over 800 members of human GPCRs are encoded and divided into six classes by sequence homology and functions. All GPCRs form a common seven-helix transmembrane embedded in the cell membrane. Although the seven transmembrane helix (TM) sequence identity is low, the 3D structure and functional mechanism are very conserved.[2] Despite the desperate demand for GPCRs, a small portion of receptors are employed as therapeutic targets. This is due to the remaining difficulty of solving and analyzing the structure of GPCRs.

GPCR drugs are classified into four types: agonists, neutral antagonists, partial agonists, and inverse agonists, and they bind to orthosteric sites of receptors with similar interactions.[3] One of the huge efforts in GPCR drug discovery has been devoted to understanding the differentiation of ligands that bind at the same site. After a ligand binds to a receptor, it causes conformational changes. Many researchers have tried to unravel this mechanism, and various consensuses have been recommended by finding conformational changes of receptors experimentally[4–9] and computationally[10–12].

Investigating the conformational changes of GPCRs has been boosted by GPCR structure determination through crystallogra-
phy.[13,14] A number of unique receptor complexes at atomic resolution is now available for over 110 GPCRs.[15] Based on the crystal structure, the importance of GPCR dynamics was emphasized and has been studied for the last decade. Not limited to crystallography, spectroscopic experiments and computer simulations have revealed the detailed mechanism of GPCRs. In particular, atomistic molecular dynamic (MD) simulations have become a more powerful tool thanks to increased computing power, improved simulation algorithms, and refined force-field parameters. [6,11,16,17] However, detecting the changes in the conformational ensemble comes with extensive experiments or large computations of more than a few hundred-microsecond protein ensemble dynamics of MD simulations.[6,7,11,17–20] The next problem is that sufficient time is needed for analysis to find proper information from the MD simulation. Thus, drug screening from ligand libraries using conformational ensemble changes has been a challenging task.

Here, to overcome the huddles and find the specificity of GPCR ligands, MD simulations and 3-dimensional residual networks (3D-ResNets) [21–23] were integrated to train the screening model. The idea was that the same type of ligand shares the same residual dynamics, which is able to show spatiotemporal patterns. Since the selective movement of the residues in the binding site induces a change in the conformational ensemble of the entire GPCR system, by capturing the pattern from the specific type of ligand, classification of the ligand type could be possible by categorizing the patterns. The spatiotemporal patterns were classified using 3D-ResNets, which have been used for action recognition. The classification model was trained with the time series of the protein distance map (PDM) of β2AR. To obtain the PDM, hundreds of MD simulation trajectories of β2AR-ligand complexes were generated and turned into the time series of PDM. Fig. 1 shows the procedure of spatiotemporal residue pair pattern analysis.

This article is organized as follows. Section II describes the materials and the simulation methods. Section III presents the details of the trained models and the spatiotemporal patterns of each ligand-bound β2AR structure. Section IV discusses the results in the context of previous computational and experimental studies.

2. Materials and methods

2.1. 3-D Convolution neural networks

3D-ResNet — A 3-Dimensional Residual Network, 3D-ResNets [21–23] was used as the ligand classification model. The 3D-ResNets is based on ResNets.[24] ResNets introduce shortcut connections that bypass a signal from one layer to the next. The connections pass through the gradient flows of networks from later layers to early layers, and ease the training of very deep networks. The difference between 3D-ResNets and original ResNets is the number of dimensions of convolutional kernels and pooling.[21]

The network architecture of 3D-ResNets in Table 1.

The residual blocks are presented in brackets. Each convolutional layer is followed by batch normalization [25] and ReLU. [26] The sizes of convolutional kernels were 7 × 7 × 7 for Conv1 and 3 × 3 × 3 for Conv2-5, respectively. The temporal stride of Conv1 is 1.[27] The network uses 1–30 frame RGB clips as inputs. The sizes of input clips were 3 channel × 1–30 frame × 312 pixel × 312 pixel. Down-sampling of the inputs were conducted by Conv3_1, Conv4_1, and Conv5_1 with a stride of 2. If the number of feature maps increased, the identity shortcuts with zero-padding was adopted to avoid increasing the number of parameters.[21,24] The dimension of the last fully-connected layer was set depending on the number of ligand type in each models.

To train 3D-ResNets, it randomly generated training samples from a time series of PDMs in training data for data augmentation. A temporal position of the MD trajectory was selected by uniform sampling to generate a training sample, and a 1- to 30-frame clip was generated around the selected temporal position. Various frames were tested for all models to obtain the best accuracy. After selection of the temporal position, a spatial position was randomly selected from the four corners or the center.[28] For multiscale cropping, a spatial scale of the sample was selected using the same procedure as in the previous works of Wang et al. [29] and Hara et al.[21] The scale was selected from \( \{1, \frac{7}{8}, \frac{7}{8}, \frac{7}{8}, \frac{7}{8} \} \). same as Hara et al.[21] did since our models were trained only in the code. The multiscale cropping is very effective for improving the performance of object recognition on the ImageNet dataset [29] Wang et al.[28] and Hara et al.[21] applied the method to action recognition with their own scale parameters to get better performance. The aspect ratio of cropped frame was 1 and the generated samples were horizontally flipped with 50% probability.[21] All generated samples had the same ligand type class labels as their original time series of PDMs. In this work, the three models—Model-3, Model-4, and Model-10 were trained to classify the residue pair pattern of the ten β2ARs into three, four and ten classes, respectively. First, Model-3 was trained to classify the binding of agonists and neutral antagonists including the apo form. Model-4 was trained with the extended training set, including the inverse agonist-bound β2AR MD trajectory, to classify the binding of the inverse agonist. Model-10 was trained to classify the binding of nine ligands plus the apo form. The training time of Model-3, 4, and 10 (3, 4, and 10 classes) of 500 epochs was around 24 ~ 48 h using two NVIDIA® TITAN RTX™ GPUs. As increasing the number of classes, sample duration, and layer depth, the training time was increased.

2.2. Preparing training Dataset

Molecular Docking—The docking simulation was performed with Schrodinger’s Glide.[30–32] The receptor grid was generated from the refined X-ray crystal structure of the alprenolol-bound inactive state of β2AR (PDB: 3NYA) in GPCRdb.[15] The same binding site of the ligand in the crystal structure was set in the grid model. The extra precision docking protocol was used, and a maximum of five poses were kept in the initial docking stage. With the obtained docking poses, MM-GBSA binding energy calculations were performed using Schrodinger’s Prime module [33] to evaluate the binding affinity of each pose. The MM-GBSA approach is one of the most widely used methods to quantify the energetic contributions of biomolecular complexes.[34] Considering the MM-GBSA binding energy and the root mean square displacement between the ligand in the X-ray crystal and the docking model, the initial structures of the β2AR-ligand complex for MD simulation were prepared.

Molecular Dynamics Simulation—The training dataset was prepared using the MD simulation trajectories of β2AR. The MD systems were constructed with the refined X-ray crystal structure of β2AR (PDB: 3NYA) docked with various ligands, as shown in Table 2. We prepared each system for simulation using CHARMM-GUI (https://www.charmm-gui.org).[35–39] All simulations were performed with GROMACS 2020.1.40–44] The CHARMM36m [45] force field was used for protein parametrization. Ligands were modeled with the CHARMM General Force Field (CGenFF).[46] The protonation states of Asp79, Glu122, Glu125, and Glu130 were neutralized, and His172 was protonated to describe the deactivation state.[11,47] The palmitoyl group was attached to Cys314.[47] The structures were solvated using the TIP3P water model [48] with Lennard–Jones interactions on hydro...
Nine ligands and one apo form were prepared for the GPCR ligands in the training set. The network architecture of 3D-ResNets with 10, 18, and 34 layers. All systems were minimized for around 0.2 h/ns using the LINCS algorithm. PyMol [55] was used to visualize and analyze the trajectories and the residue pair patterns. The performance of the MD simulation per ligand was around 0.2 h/ns using 4 × 20 cores of 2.40 GHz Intel® Xeon® Gold 6148 processor. Based on the hyperparameter test, at least 200 MD trajectories (150 for training and 50 for validation) are needed for training single ligand. Thus, it takes 400 h/ligand to obtain the dataset of 10 ns × 200 MD trajectory, approximately. Using GPUs will greatly reduce the MD simulation time.

Protein Distance Map—A protein distance map (PDM) represents the distance between all possible amino acid residue pairs of a 3-dimensional protein structure using a binary 2-dimensional matrix. Here, we generated a PDM for every MD trajectory for the training data. In Fig. 2, for two residues i and j, the ij element of the matrix is the distance between residue i and j. The X- and Y-axis represent the residue index from 1 to 312 which is the same number of residues in the β2AR structure used in the MD simulation.

| Ligand Name | Type | PDB ID | RMSD (Å) |
|-------------|------|--------|----------|
| BI-167107   | Agonist | 3POG  | 0.177    |
| Epinephrine | Agonist | 4LDO  | 0.072    |
| Salmeterol  | Agonist | 6MXT  | 1.668    |
| Alprenolol  | Neutral Agonist | 3NYA | 0.000    |
| (S)-Propranolol | Neutral Agonist | 6P55 | 0.197    |
| Timolol     | Neutral Agonist | 6PS6 | 0.428    |
| Carazolol   | Inverse Agonist | 2RH1 | 0.196    |
| ICI-118551  | Inverse Agonist | 3NY8 | 0.293    |
| Q27461782   | Inverse Agonist | 3NY9 | 0.409    |

RMSD: Root mean square displacement between the ligand in the X-ray crystal and the docking model.
tions and each pixels represents the minimum side-chain distance between the pair of residues. The color gradient below shows the distance. In this work, the smallest distance between two residues was calculated using the GROMACS command gmx mdmat. After obtaining the PDM matrix, the output was processed with gmx xpm2ps to make an image file of PDM. The 14 residues in highly flexible intracellular loop 3 were omitted.

2.3. Attention map

M3d-CAM is an easy-to-use library for generating attention maps of CNN-based PyTorch models improving the interpretability of model predictions for humans. Attention maps can be generated with multiple methods, such as guided backpropagation, Grad-CAM, guided Grad-CAM and Grad-CAM++. These attention maps visualize the regions in the input data that influence the model prediction the most at a certain layer. Furthermore, M3d-CAM supports 2D and 3D data for the task of classification as well as for segmentation.[56] A key feature is also that in most cases, only a single line of code is required for generating attention maps for a model, making M3d-CAM basically plug and play. After generating the attention maps, NiBabel [57] and ImageJ [58] were used to analyze and visualize the attention map of the models.

3. Results and discussion

3.1. Tuning hyperparameters

In this work, the three models—Model-3, Model-4, and Model-10—were trained, and their accuracies were evaluated. The details of each model are shown in Table 3. Models 3, 4, and 10 were trained to classify the residue pair pattern of the ten β2ARs into three, four and ten classes, respectively. First, Model-3 was trained to classify the binding of agonists and neutral antagonists and the apo form. Model-4 was trained with the extended training set, including the inverse agonist-bound β2AR MD trajectory, to classify the binding of the inverse agonist. Model-10 was trained to classify the binding of nine ligands and the apo form.

To acquire an optimized model with higher accuracy, three important hyperparameters—the sample duration, the model depth, and the size of the training set—were tuned. The tuning was performed in sequence of the sample duration test, the model depth test, and the size of the training set test. The following test was carried out with the optimized hyperparameter in the previous test. A total of 500 epoch training runs were performed for every case. At 100, 200, and 300 epochs, multistep learning rates of 0.1, 0.01, and 0.001 were applied. First, the sample duration test started with a model depth of 10 layers, and the size of the training set is shown in Table 3.

In Fig. 3, the results of the sample duration test are presented. The sample duration is the number of input frames of the time series of the PDM in the training set. The frame is randomly cropped from a time position in the MD trajectory. Each 10-ns MD trajectory consists of 100 PDM frames; therefore, the interval between adjacent frames is 0.1 ns. Since a previous study has shown that a longer temporal duration improves recognition accuracy,[59] the sample duration was tested from 1 to 30 frames (0–3 ns). More than 30 frames were also performed; however, it was difficult to obtain consistent training results due to GPU memory limits. Three training runs were performed for each sample duration frame, and the optimal frame of each model was selected for further training and analysis. In Fig. 3(A), Model-3_1 with 15 frames gave the best accuracy of 0.855 among all models. Fig. 3(B) shows that the train-

![Fig. 2. An example of a protein distance map of β2AR from the MD trajectory. The X- and Y-axis represent the residue index from 1 to 312 and each pixels represents the minimum side-chain distance between the pair of residues. The color scale below shows the color gradient from a distance of 0 to 5 nm.](image-url)
ing of Model-4 did not achieve good accuracy for all frame cases. Model-4, with 25 frames gave the best accuracy of 0.622, which is not enough performance to classify the inversed agonist. In Fig. 3 (C), Model-10, with 30 frames gave the best accuracy of 0.833 among all models. Unlike previous research on human action recognition,[59] the results showed that the sample duration did not dramatically improve the accuracy. Even in the Model-3 case, 15 frames showed higher accuracy than 20–30 frames. We presumed that the model training is greatly affected by the random temporal cropping point of the time series of the PDM. A ligand-induced specific residue pair pattern might not be maintained during the whole 10-ns MD trajectory. Therefore, the random cropping point could not capture the moment. Random cropping could be improved by focusing on specific temporal moments and residues in further studies. In the next test, the sample duration frames—15 frames for Model-3, 25 frames for Model-4, and 30 frames for Model-10—were used since they showed the best accuracy in each model.

Next, the model depth was varied by changing the number of layers of the 3D-ResNet architecture. The three architectures, ResNet-10, ResNet-18 and ResNet-34 in Table 1, were tested with the same training set in the sample duration test. Basically, the deeper the layer is, the higher the accuracy of the model in action recognition.[22] However, in Fig. 4, the 10-layer model showed higher accuracy than the 18- and 34-layer models. Since the deeper the layer, the more parameters in the network need to be trained, for the model with few classes in this study, 10 layers were enough to obtain high accuracy for Models-3 and –10. Model-4 did not show any improvement for the sample duration test.

The last test was finding the optimal size of the training set, and Model-3 with a 15-frame sample duration and 10-layer depth was used for the test. The test was performed by decreasing the num-

| Table 3 |
The classes in each model and their details. |

| Class                  | Train | Val/Test | Class                  | Train | Val/Test | Class                  | Train | Val/Test |
|------------------------|-------|----------|------------------------|-------|----------|------------------------|-------|----------|
| Agonist                | 450   | 150      | Agonist                | 450   | 150      | BI-167107              | 150   | 50       |
| Neutral antagonist     | 450   | 150      | Neutral antagonist     | 450   | 150      | Salmeterol             | 150   | 50       |
|                        |       |          |                        |       |          | Epinephrine            | 150   | 50       |
|                        |       |          |                        |       |          | Alprenolol             | 150   | 50       |
|                        |       |          |                        |       |          | (S)-Propranolol        | 150   | 50       |
|                        |       |          |                        |       |          | Timolol                | 150   | 50       |
| Inverse agonist        |       |          | Inverse agonist        | 450   | 50       | Carazolol              | 150   | 50       |
|                        |       |          |                        |       |          | ICI-118551             | 150   | 50       |
| Apo form               | 150   | 50       | Apo-form               | 150   | 50       | Q27461782              | 150   | 50       |

| Fig. 3. The averaged accuracies of the validation set for the 10-layer depth Model-3, 4, and 10 with sample durations from 1 to 30 frames shown in (A), (B), and (C). The X- and Y-axis represent the sample duration and the trained models’ accuracy, respectively. The sample duration was presented in the unit of frame in which the interval is 0.1 ns in the MD trajectory. The averaged accuracy was calculated with the last 50 epochs of the training. Each model was trained three times with the same training set with different temporal cropping points, and the results of the models are shown by blue, pick and orange lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) |
number of MD trajectories of each ligand in the training set from 150 to 3. Fig. 5 showed increasing accuracy depending on the number of MD trajectories in the training set. For the human action dataset, Kinetics,[60] as the number of video clips per class increased, the top-1 and top-5 accuracies were improved. Fig. 5 showed a similar result as for the Kinetics dataset. Even though the convergence of accuracy could not be achieved as a limitation of computing cost, we found that the 150 MD trajectories were enough to train Model-3. We speculated that Model-4 might be improved by increasing the number of MD trajectories in further studies.

3.2. Training curve and model accuracy

In Fig. 6, the training and validation curves of the models with optimal hyperparameters are presented. A total of 500 epoch runs were performed for each training. At 100, 200, and 300 epochs, the training curve showed a change in the gradient because of the multistep learning rates—0.1, 0.01, and 0.001. Models-3 and -10 showed relatively high convergence accuracy near 0.8, but Model-4 showed lower accuracy near 0.6. These results showed that the training of Models-3 and -10 was successful but not that of Model-4. In Table 4, the averaged accuracies of the last 50 epochs are presented. The definition of the accuracy in this work is like below:

\[
\text{Accuracy} = \frac{\text{Number of MD trajectories with correct top} - 1 \text{ class prediction}}{\text{Total number of MD trajectories in the class}}
\]

The test set was prepared with exactly the same system as the training and validation set except for the initial atomic velocity distribution. Model-3 showed the highest accuracy among the three models. This represents the classification of the agonist, neutral antagonist, and apo form was successful with the test set. The relatively high accuracy of Model-10 showed good performance of the model in the classification of each ligands. This result shows that Model-10 could recognize the difference residual patterns.

For more detail analysis of the model’s performance, the individual ligand and class prediction accuracy of each model were presented in Fig. 7. Model-3 shows the individual ligand accuracy above 0.85 for all ligands. Epinephrine shows the highest prediction accuracy of 1.00. Model-4 shows relatively low accuracy of BI-167107, (S)-Propranolol, and ICI-118551. The agonist prediction performance was poor than the neutral antagonist and inverse agonist. Interestingly, the prediction rate of the apo form was zero which mean no predictions were correct. In Figure S12, the inverse agonist scores the most for almost trajectories, which represents the model couldn’t discriminate the spatiotemporal pattern of the apo form and the inverse agonist. Model-10 shows the individual ligand accuracy above 0.84 for all ligands except ICI-118551 which also shows lower accuracy in Model-4. This result indicates that the dataset preparation needs to be done with ligand specific manner for improving the screening performance. To improve the performance, the training set of the ligands with low accuracy needs to be increased and varied for further development. For detailed analysis of the scoring of each trajectory in the test set, the ligand scores and accuracy of each ligand in Model-3 and 4 were evaluated in Figs. 8, 9 and Figure S9-S13 were evaluated.
Fig. 8 and Fig. 9 show the Model-3 evaluated ligand type score of each MD trajectory in Test set-1. Fig. 8 shows the structure and scores of the three agonists. All three agonists showed good Top-1 accuracy. For almost all MD trajectories, Model-3 classified the ligands as agonists. Six MD trajectories of BI-167107 and salmeterol had higher antagonist scores than agonist scores, and the accuracy was 0.88. In the epinephrine case, all the MD trajectories were recognized as agonists, and the accuracy was 1.00. Fig. 9 shows the structure and scores of three neutral antagonists. Almost all three neutral antagonists were classified as neutral antagonists. Two MD trajectories of alprenolol and (S)-propranolol had higher agonist scores than neutral antagonist scores, and the accuracy was 0.96. Three MD trajectories of timolol showed higher agonist scores than neutral antagonist scores, and the accuracy was 0.95. The results implied that the residue pair pattern of the apo form is clearly classified from the agonist and neutral antagonist. For a more detailed analysis of the residue pair pattern, the attention map of each test set was investigated.

3.3. Attention map analysis

The attention maps were generated by M3d-CAM [56] from the last layer, Cov_5x in Model-3. The analysis was carried out only for

| Model name | Validation set | Test set |
|------------|----------------|---------|
| Model-3    | 0.855          | 0.923   |
| Model-4    | 0.622          | 0.688   |
| Model-10   | 0.833          | 0.886   |

Fig. 7. The individual ligand and class prediction accuracy of Model-3, 4 and 10 of the Test set. In the Model-3 and 4 case, the individual ligand prediction accuracies were evaluated from the correct prediction of Top-1 accuracy of each ligand. In Model-10 case, only the individual ligand prediction was presented since the classification was performed only for the individual ligand, not for the ligand type.
Model-3 since the model caught some different patterns between the agonists and neutral antagonist. The patterns from Model-10 were too complex to separate types of ligand. The obtained attention maps were analyzed by using NiBabel [57], ImageJ [58] and in-house Python code. The number of attention maps was more than 100 frames per MD trajectory. Depending on the sample duration, the total frame of the attention map was determined. For example, if the sample duration was 15 frames, after one M3d-CAM run, seven sets of attention maps were generated. Each set consists of 15 frames, which is the same as the sample duration. Thus, a total of 105 attention maps were obtained from one 10-ns MD trajectory. If the sample duration was 25 or 30, the number of obtained attention maps would be 100 or 120, respectively. After the generation of the attention map from all the MD trajectories, to find the most significant residue pair pattern, the brightest pixel of each attention map was collected. Fig. 10 shows an example of the pattern collecting procedure. Since each pixel in the attention map stands for every residue pair, collecting the brightest pixel would be the most significant pattern that the model could recognize. The first 10 ns of the MD production run was neglected to prevent an unwanted effect from the starting conformation. All the collected patterns were projected on the 3-dimensional structure of β2AR, and some noticeable patterns are presented in the next section.
3.4. Residue pair pattern analysis – Apo form

The three significant residues and their pairwise patterns of the apo form of β2AR are illustrated in Fig. 11. The residue number of the β2AR is presented with the combination of the PDB file residue numbering and the sequence-based generic GPCR residue numbering scheme of class A (Ballesteros—Weinstein). In this system, the residue type and number in the PDB file is presented with the two numbers of superscript. The first number represents the helix and the second the residue position relative to the most conserved position, which is assigned the number 50. For example, V1143.33 denotes the Valine 114 located in TM3, 17 residues before the most conserved residue, R1313.50. To identify the significant residues, all the pairwise patterns were evaluated and projected on the β2AR structures in the Supporting Information. Since analyzing all the patterns was too complicated, we defined the three significant residues with the highest counts in the pairwise patterns. The residue pairs in this investigation represent their noticeable spatiotemporal pattern from the time series of the PDMs. In the apo form in Fig. 11, the three significant residues were Ile1594.61 in TM4, His2696.31 in TM6 and Phe240 in intracellular loop 3 (ICL3). The pair residues of Ile1594.51 were located in various other domains—TM1, TM2, TM3, ICL1, and ICL3. Interestingly, His2696.31 in TM6 was correlated with the residues in TM3 near the ligand binding site. Model-3 recognized the apo form dynamics by focusing on the spatiotemporal patterns between the binding site and TM6, which plays a major role in the activation/deactivation of β2AR. Phe240 in ICL3 showed some patterns with the residues in TM3, TM4 and extracellular loop 2 (ECL2). Since the conformation of ICL3 is highly correlated with the bias signal of...
β2AR [63], ICL3-associated dynamics might be one of the key patterns to classify the signals. However, the 14 residues in ICL3 were omitted in this work, and the ICL3-associated patterns might be artificial in this model. We envisioned that using the full structure of β2AR in further research provides more information on ICL3 dynamics.

3.5. Residue pair pattern analysis – Agonists

Without agonists, the ground state conformation of the receptor is maintained by a network of intramolecular contacts that prevent conformational changes. Upon binding agonists, these restraining interactions are broken, and alternative interactions emerge that
facilitate the formation of the active receptor conformation.\[64]\] These alternative interactions were indirectly illustrated by capturing the significant residues and their pairwise patterns of the three agonists bound β2AR, as shown in Fig. 12. The prominent difference from the apo form was the significant residue in the binding site. The BI-167107-bound case in Fig. 12(A) shows that Ile1123.31 in the binding site has a pairwise pattern between the residues in ICL3 and the cytoplasmic ends of TM6. In particular, the residues in TM6—Lys2706.32, Ala2716.33, and Lys2736.35—were located in the cytoplasmic region, which shows outward movement in active β2AR. This indicates that the binding of the ligand directly alters the movement of the residues in the binding site and that the binding site residues indirectly induce the dynamics of the residues in TM6. Similar patterns between the binding site and TM6 were shown in the Lys2706.32 residue case. Lys2706.32 had paired residues in the ligand binding site. This means that Model-3 captured the correlation between the ligand binding site and the TM6 region by recognizing the residue pair dynamics within 10 ns. This result showed that 3D-ResNets could analyze spatiotemporal patterns that cannot be captured by conventional molecular dynamics analysis methods. Similar to the pattern in the apo form, Phe240 in ICL3 showed some patterns with the residues in TM3, TM4 and ECL2.

The salmeterol-bound β2AR residue pair patterns were projected on the β2AR structure in Fig. 12(B). Similar to BI-167107, salmeterol showed noticeable patterns near residues Ile1123.31, K2706.32 and Lys270. Similar paired residues were associated with those residues in the binding site, TM6, and ICL2. On the other hand, epinephrine in Fig. 12(C) showed different patterns in A1193.38 and His1724.64. As epinephrine is not a β2AR selective agonist, it might show a different pattern compared to the others. Model-3 succeeded in classifying the epinephrine bound pattern despite the different significant residues, so somehow the 3D-ResNets could capture the pattern, which was not determined via attention map analysis.

3.6. Residue pair pattern analysis – Neutral antagonists

The significant residues and their pairwise patterns of the neutral antagonist bound β2AR are illustrated in Fig. 13. The most pattern counted residues in Fig. 13(A), the alpronisol-bound β2AR case are in Val1143.31, K2706.32 and Lys270. Similar paired residues were associated with those residues in the binding site, TM6, and ICL2. There was a pairwise pattern between the residues in TM4, ECL2, ICL3 and TM6. Similar to the agonist cases, the residues in the cytoplasmic end of TM6 showed patterns between the binding site residues. In addition to these patterns, the involvement of the
residues in ECL2 was determined. ECL2 has been revealed as the essential region that contributes to receptor activation and ligand binding of the family A GPCR.[64] Although the ECLs in the β2AR crystal structure of an active conformation are very similar to the ECLs in an inactive conformation, Model-3 captured different dynamic patterns between the binding site residue and ECL2. Two other neutral antagonists in Fig. 13(B) and (C) showed similar residue pairs between Val114 and ECL2. Another significant residue was Lys270 in the cytoplasmic end of TM6. In all three cases, Lys270 was linked to the residues near the ligand binding site. This implies that the cytoplasmic end of TM6 is closely related to the ligand binding site, similar to that in the agonist-bound cases. The last significant residue was Trp173 in the alprenolol- and (S)-propranolol-bound cases in Fig. 13(A) and (B) and Val126 in the timolol-bound case in Fig. 13(C). Trp173 is located in the ECL2 region and has some pair residues in TM3 near the ligand binding site. Again, this confirmed that ECL2 is highly related to the ligand binding site in neutral antagonist-bound receptors. The timolol-bound case showed residue pairs in TM2, ECL2, and ICL3. In all three cases, the patterns associated with F240 in ICL3 were also counted but not predominant compared with the other patterns. All the patterns and some significant residues are found in the Supporting Information.

Fig. 13. The three significant residues and their pairs are presented on (A) the Alprenolol, (B) the (S)-Propranolol, and (C) Timolol bound β2AR structures. The thickness of the tube represents the number of pair patterns with which the residue in the position is associated. The pair residues of each significant residue are listed in the tables below the snapshots. The dashed lines in blue connect the relative residue pairs with each other. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

residues in ECL2 was determined. ECL2 has been revealed as the essential region that contributes to receptor activation and ligand binding of the family A GPCR.[64] Although the ECLs in the β2AR crystal structure of an active conformation are very similar to the ECLs in an inactive conformation, Model-3 captured different dynamic patterns between the binding site residue and ECL2. Two other neutral antagonists in Fig. 13(B) and (C) showed similar residue pairs between Val114 and ECL2. Another significant residue was Lys270 in the cytoplasmic end of TM6. In all three cases, Lys270 was linked to the residues near the ligand binding site. This implies that the cytoplasmic end of TM6 is closely related to the ligand binding site, similar to that in the agonist-bound cases. The last significant residue was Trp173 in the alprenolol- and (S)-propranolol-bound cases in Fig. 13(A) and (B) and Val126 in the timolol-bound case in Fig. 13(C). Trp173 is located in the ECL2 region and has some pair residues in TM3 near the ligand binding site. Again, this confirmed that ECL2 is highly related to the ligand binding site in neutral antagonist-bound receptors. The timolol-bound case showed residue pairs in TM2, ECL2, and ICL3. In all three cases, the patterns associated with F240 in ICL3 were also counted but not predominant compared with the other patterns. All the patterns and some significant residues are found in the Supporting Information.
4. Conclusion

GPCRs are promising drug targets because they play a large role in modulating diverse signaling pathways in the human body. The signaling pathways are regulated by agonists, inverse agonists, and neutral antagonists. Upon binding each type of ligand, signaling is changed by shifting the conformational ensemble of the GPCR. Detecting the changes in the conformational ensemble comes with time- and cost-consuming experiments or large computations of MD simulations. To overcome these obstacles, we investigated the conformational changes by capturing the spatiotemporal residue pair pattern of the ligands bound β2AR using 3D-ResNets trained with a time series of PDMs extracted from the MD simulation trajectories. The idea was that the same type of ligand shares the same residual dynamics as spatiotemporal patterns. The specific movement of the residues in the binding site might induce a change in the conformational ensemble of the entire system. Thus, by capturing the pattern from the specific type of ligand, classification of the ligand type could be possible. The spatiotemporal patterns were classified using 3D-ResNets, which have been used for action recognition. The models were trained with the time series of the PDMs. To obtain the PDMs, a few hundred 10-ns MD simulation trajectories of β2AR-ligand complexes were generated and transformed to the PDMs.

Three hyperparameters—sample duration, model depth, and the number of MD trajectories in the training set—were tested, and it was found that the number of MD trajectories significantly affected the model’s accuracy. During the test, the classification of agonist and neutral antagonist was successful with Model-3, but that of the inverse agonist was not. Different residue pair patterns were spotted between the agonists and neutral antagonists.

For detailed analysis of the residue pair pattern, the attention map of each test set of Model-3 was investigated. For each ligand, three significant residues were defined, and all the pairwise patterns were evaluated and projected on the β2AR structures. The residue pairs in this investigation represent their noticeable spatiotemporal pattern from the time series of the PDMs.

Interestingly, Val1143.33 was in the ligand binding site, and Lys270 and His269 were in the cytoplasmic end of TM6. This implied that the residues in the binding site were indirectly linked to the intracellular side of TM6, whose outward motion is related to the activation of β2AR.[11] Model-3 could recognize the side-chain pattern between the agonist binding site and the intracellular region of TM6 within a 10-ns MD trajectory. Since the activation and deactivation of β2AR were classified with the shape of TM6 around the connector region, the recognition of residue pair patterns between the binding site and the connector region is reasonable. A recent study on the shortest pathway analysis of β2AR showed a similar dynamic network of the residues between the binding site and TM6.[64] We assumed that 3D-ResNets recognized the dynamical network of the residues as a pairwise pattern of the sidechains so that they gave similar results.

All three neutral antagonists showed residue pair patterns of Val1143.33-Arg175 (ECL2) and Val1143.33-Lys2706.32. The patterns between the residues in the ligand binding site and ECL2 were not found in the agonist-bound cases. ECL2 of the class A GPCR has been studied as the essential region that contributes to the activation and ligand binding of class A GPCRs.[64] The results showed that the binding site residue Val1143.33 was indirectly linked to ECL2 near Arg175 and altered the residue pair patterns. In the agonist-bound case, the residues in ECL2 and ICL3 were also found in patterns; however, the patterns with the binding site residue were not significant. Model-3 could not capture any patterns in TM7, which shows the inward movement of the NPxxY motif during GPCR activation.[16] The summary of the number of pattern distribution over all residues in the seven β2AR cases are presented in Fig. 14.

To compare the model’s ligand classification performance with the previous method, the ligand docking and MM-GBSA binding energy calculation were performed. Previously, Hui et al.[47] calculated binding free energy of the three known β2AR ligands—BI-167107, alprenolol, and carazolol—using free energy perturbation (FEP) method and found that the agonist (BI-167107) was bound stronger to the active form of β2AR than the inactive one. The other
way, the neutral antagonist (alprenolol) and inverse agonist (carazolol) showed stronger binding free energy in the inactive form than the active one. In the same way, the MM-GBSA binding energy were used as the control method to classify the nine ligands. Figure S8 shows the MM-GBSA binding energy of the nine ligands bound with the active and inactive form of β2AR. First, the ligand docking was performed with the two different receptor structures—the active (PDB ID: 3POG) and inactive (PDB ID: 3NYA) forms and the binding energy was calculated with their docking poses. The agonists, BI-167107 and epinephrine show the stronger binding to the active form than the neutral antagonists, alprenolol and (S)-propranolol. However, the agonist, salmeterol and neutral antagonist, timolol show stronger binding to the inactive and active form, respectively. The results indicate that the MM-GBSA binding energy couldn’t classify the agonist and neutral antagonist, clearly.

As the 3D-ResNet classification mechanism was too complicated to interpret, we could not show a clear view of dominant patterns depending on the bound ligands. However, as our best effort to interpret the attention map, we focused on the residues that contributed more to the patterns. Previously, the conformational changes of loosely coupled three key regions—the binding pocket, the connector region, and the G protein-binding site—were suggested to explain the activation/deactivation mechanism of β2AR.[11] Similar to the large-scale MD simulation result, those three regions were appeared in the residue pattern analysis in Fig. 13. The binding pocket region is around V114 in TM3, the connector region near K270R in TM6, and the G protein-binding site is near F240 in ICL3. The three regions showed slightly different position compared to the previous work because they used an active β2AR as a starting structure. Although, the 3D-ResNet could capture the patterns of the loosely coupled three regions related to the signal transmitting pathway without the large-scale MD simulations. Basically, 3D-ResNets considers and recognizes all the patterns simultaneously; thus, this kind of analysis might not be sufficient to solve the black box of the recognized residue pair patterns upon ligand binding. In further studies, principal component analysis of the significant residues in the MD trajectory may help to reveal the residue pair pattern and the functional dynamics of the β2AR-ligand complex. Recognition of the inverse agonist pattern might be possible by extending the training set with various MD systems since the working mechanism of the inverse agonist is determined by the basal activity of GPCRs.[65] With the help of binding free energy calculations, such as the free energy perturbation method, the trained model would work as a good screening tool for GPCR drugs. This work demonstrated the potential application of the 3D neural network in drug screening, as well as in protein functional dynamics analysis.

As a drug screening tool, this version is incomplete one because the negative data set, such as decoys[66,67] were not integrated in the training set. Thus, if a different ligand type were used as the test set, the model will predict it as one of the ligand types in the classes which scores the most, even though the ligand is none of them. In this work, however, we would rather focus on the residue pair pattern analysis since the huge computational time will be needed to include the decoys. In further research, well-balanced decoy set will be integrated for developing a drug screening tool.

5. Data and software availability

The 3D-ResNets and the MD calculations were performed with the publicly available free open source code. The docking and MM-GBSA calculations were performed with Schrodinger’s Glide and Prime. The protein–ligand structures, GROMACS input files and attention map analysis Python code are available at https://github.com/MinwooHan84/beta2AR.git.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was funded by the National Research Foundation of South Korea, grant number NRF-2019M3E5D4065299. The simulations were carried out with the supercomputer NURION in the National Supercomputing Center of the Korea Institute of Science and Technology Information (KISTI) of South Korea.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2022.10.036.

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