Subject Section

DeepREAL: A Deep Learning Powered Multi-scale Modeling Framework for Predicting Out-of-distribution Ligand-induced GPCR Activity

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Abstract

Motivation: Drug discovery has witnessed intensive exploration of predictive modeling of drug-target physical interactions over two decades. However, a critical knowledge gap needs to be filled for correlating drug-target interactions with clinical outcomes: predicting genome-wide receptor activities or function selectivity, especially agonist vs. antagonist, induced by novel chemicals. Two major obstacles compound the difficulty on this task: known data of receptor activity is far too scarce to train a robust model in light of genome-scale applications, and real-world applications need to deploy a model on data from various shifted distributions.

Results: To address these challenges, we have developed an end-to-end deep learning framework, DeepREAL, for multi-scale modeling of genome-wide ligand-induced receptor activities. DeepREAL utilizes self-supervised learning on tens of millions of protein sequences and pre-trained binary interaction classification to solve the data distribution shift and data scarcity problems. Extensive benchmark studies on GPCRs, which simulate real-world scenarios, demonstrate that DeepREAL achieves state-of-the-art performances in out-of-distribution settings. DeepREAL can be extended to other gene families beyond GPCRs.

Availability: All data used are downloaded from PfamMistry et al., 2020, GLASSChan et al., 2015 and IUPHAR/BPS and the data from referenceSakamuru et al., 2021. Readers are directed to their official website for original data. Code is available on GitHub https://github.com/XieResearchGroup/DeepREAL.

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Supplementary information: Supporting figures are available at Bioinformatics online.

1 Introduction

Over the last two decades, drug discovery has been dominated by target-based high-throughput compound screening. Unfortunately, this “one-drug-one-gene” approach has been costly and had a low success rate due to our limited understanding of molecular and cellular mechanisms of drug actionsDiMasi et al., 2016Wong et al., 2019. Drugs from the target-based screening often interact with unexpected off-targets, leading to serious side effectsLin et al., 2019Lynch III et al., 2017. Furthermore, a polypharmacology approach is often needed to achieve desired therapeutic efficacy and overcome drug resistance for complex diseasesXie et al., 2012. In order to predict drug phenotypic response at the organismal level, it is necessary to not only elucidate genome-scale drug-target interactions (DTIs) but also reveal how DTIs collectively modulate a biological system.
DeepREAL

The drug mode of action is a multi-scale process that starts with drug binding to its targets, principally proteins. Then the drug can act as an antagonist or an agonist to block or enhance downstream biological processes, respectively. Therefore, it is critically important to model the change of receptor activities or functional selectivity upon the drug binding for understanding how the drug modulates pathophysiological functions. The information on the receptor activity following the ligand binding will fill in a critical knowledge gap in correlating DTIs to clinical outcomes.

Although a great deal of efforts have been devoted to predict genome-wide DTIs using deep learning, few large-scale experimental and computational studies have been able to specify the ligand-induced receptor activity, i.e., the functional selectivity of the ligand as an antagonist or an agonist. Although a great deal of efforts have been devoted to predict genome-wide DTIs using deep learning, few large-scale experimental and computational studies have been able to specify the ligand-induced receptor activity, i.e., the functional selectivity of the ligand as an antagonist or an agonist. Sakamuru et al., 2021.

In this research, we aim to predict not only whether any pairs of proteins and chemicals interact with each other or not but also the receptor activity upon the binding, especially, making reliable predictions for understudied “dark” proteins that do not have any ligand annotations. Only a limited number of receptors have sufficient function selectivity data to train a robust machine learning model. Thus, the one-protein-one-model approach cannot be extended to majority of proteins that have few or no labeled data. Sakamuru et al., 2021. An early work applied a neural network model to predict multiple interaction types for annotated proteins Wang and Zeng, 2013. However, this work neither included antagonist/agonist as prediction tasks nor was tested for dark proteins. It is a challenging task to predict the function for dark proteins in general using machine learning.

Conventional machine learning methods assume that the distribution of unseen data and training data is identically and independently distributed (IID). This assumption may not hold for the dark proteins that are dissimilar from those in the training data. In other words, many dark proteins are out-of-distribution (OOD) in terms of the training samples. Similarly, unseen novel chemicals whose structures are different from those in the training set are also OOD cases. To address the data scarcity and OOD challenges, we have developed an Artificial Intelligence (AI)-powered multi-scale modeling framework, DeepREAL, to simulate the multi-scale drug actions and predict the ligand-induced receptor activity for dark proteins and novel chemicals. We first apply self-supervised learning to train a protein sequence model for a universal protein sequence embedding on a genome scale. This allows us to detect subtle relationships between dark proteins and ligand-annotated proteins as demonstrated in other studies. Wang and Zeng, 2013; Cai et al., 2021; Rao et al., 2019; Rives et al., 2021. We then train a binary classification deep learning model to predict whether a chemical binds to a protein and extract a latent presentation of DTIs. Because there is a large amount of binary interaction data, it is possible to train a robust deep learning model. Finally, we integrate chemical embedding model, sequence embedding model, and DTI latent representation model to train an end-to-end deep learning model for predicting the ligand-induced receptor activity using limited data. In the rigorous benchmark studies on GPCRs, which simulate real-world applications, DeepREAL significantly improves the generalization ability in the OOD setting compared with the state-of-the-art methods.

The contributions of DeepREAL can be summarized in two folds:

1. DeepREAL aims to address an unsolved but important challenging problem for drug discovery: robustly predicting genome-wide ligand-induced receptor activities or function selectivity under various data distribution shifts.
2. DeepREAL is based on a new multi-stage deep transfer learning architecture that combines binary DTI pretraining and embedding with a three-way receptor activity fine-tuning to address OOD challenges using sparse receptor activity data.
2 Results and discussion

2.1 Overview of methods

Given a chemical structure and the sequence of a receptor protein, DeepREAL will predict whether the chemical is an agonist or an antagonist if it binds to the receptor, or not bind to it at all (Figure 1A). As an end-to-end learning framework, the DeepREAL is a three-way classifier: not-binding/agonist/antagonist. Intuitively, DeepREAL leverages large data sets to hierarchically inform predictions on the receptor activity whose labeled data are scarce along a three-stage pretraining-fine-tuning pipeline as illustrated in Figure 1B. In Stage 1, protein descriptor was pre-trained using PfamMoty et al., 2020 data. In Stage 2, a binary DTI classifier was then pre-trained using GLASSCan et al., 2015 and IUPHAR binary data Armstrong et al., 2019. Finally, in Stage 3, three-way classification on the receptor activity was fine-tuned using the outputs of Stage 1 and Stage 2 as inputs with IUPHAR antagonist/agonist data Armstrong et al., 2019.

The Stage 1 self-supervised sequence embedding was based on DISAE Cai et al., 2021. DISAE distilled the protein sequence into an ordered list of triplets by excluding evolutionarily unimportant positions from a multiple sequence alignment. Then long range residue interactions were learned via the self-attention in a transformer module. A self-supervised masked language modeling (MLM) approach was used to train sequence embeddings. By pretraining protein sequences on whole Pfam in Stage 1, DeepREAL equipped itself with genome-scale protein representations that captured novel relationships between proteins beyond sequence homology as demonstrated by several studies Cai et al., 2021; Rao et al., 2019; Rives et al., 2021. The second stage was a binary DTI pretraining which predicts binding/not-binding. By pretraining on a large scale of binary DTI data in Stage 2, DeepREAL builds knowledge of chemical-protein interactions which is the initial step in the ligand binding event and generates DTI embeddings. Finally, in Stage 3, information learned from sequence embeddings and DTI embeddings were transferred into predicting receptor activities using a small amount of data. This hierarchy design maintained knowledge learned from heterogeneous resources and enhanced model robustness when facing shifted data distribution during the deployment. The model was trained in an end-to-end fashion without feature engineering. The embedding from the pretraining is not fixed, but can be fine-tuned by the subsequent training stage. More details of DeepREAL design and implementation could be found in Methods section. Detailed model architecture is in Supporting Figure S1 and Table S1.

It notes that DeepREAL is an extension of DISAE Cai et al., 2021 but with several major new contributions. DISAE is a general-purpose protein language model and has been applied to predict chemical-protein interactions Cai et al., 2021, while DeepREAL is a framework designed to tackle a different task that has not been explored: predicting out-of-distribution ligand-induced receptor activity (agonist vs. antagonist). This task cannot be solved by the original DISAE architecture Cai et al., 2021. In the DeepREAL framework, DISAE was mainly used as stage 1 pretraining. In addition, DeepREAL included two more components beyond DISAE: stage 2 DTI interaction embedding and stage 3 three-way classification.

As shown in Table 1, 689 unique human GPCRs were used for the stage 2 DTI pretraining. These GPCRs consist of six Pfam families: PF00001, PF00002, PF00003, PF0052496, PF01534, and PF02101. Among them, 450 GPCRs have known labeled receptor activity data, and was used for the stage 3 fine-tuning. Among 180,000 ligands of GPCRs, only 3303 ligands have known agonist/antagonist activities. Moreover, majority GPCRs have less than 100 ligands that are labeled with receptor activities, as shown in the Supporting Figure S2. Only 3 opioid receptors (P55372 - Mu Opioid receptor, P41143 - Kappa Opioid receptor, P41145 - Delta Opioid receptor) have more than 300 chemicals with known receptor activities. Thus, the labeled receptor activity data are not large enough to train a robust machine learning model on the basis of a single protein for most GPCRs.

To evaluate DeepREAL performance in light of real-world applications for dark proteins and novel chemicals, both data preprocessing and controlled experiment are designed to simulate various scenarios of data distribution shifts and to answer the following questions.

Q1: Is the pretraining helpful to improve the performance of receptor activity prediction using a small amount of data?
Q2: When DeepREAL is applied to unseen dark proteins that have low sequence similarity to those in the training data, what is the OOD generalization performance of DeepREAL?
Q3: When DeepREAL is used to predict unseen novel chemicals that are significantly different from those in the training data, what is the OOD generalization performance of DeepREAL?
Q4: When the test set label (agonist/antagonist/not-binding) distribution is close to reality and imbalanced compared to the training data, what is the generalization performance of DeepREAL?
Q5: How does DeepREAL perform compared to the state-of-the-art baseline models in both OOD and IID settings for predicting Opioid receptor activity?

We used three metrics, AUC-ROC, MCC and Cohen’s kappa to evaluate the performance of various models under different settings.

2.2 Pretraining enables DeepREAL to generalize genome-scale receptor activity predictions using a relatively small data set

Pretraining has been demonstrated to be effective in several recent works Wan and Zeng, 2016; Karimi et al., 2019 for predicting protein-ligand interactions. DeepREAL used stage 1 and stage 2 as pretrainings for learning knowledge in the protein sequence space and binary interaction space, respectively. To answer Q1, the same model architecture is trained on the same IID and OOD settings using four procedures: 1) from total scratch without any pretraining, i.e., Stage 3 only; 2) going through Stage 1 whole Pfam pretraining but not the Stage 2 binary DTI classification pretraining, which is equivalent to the DISAE model Cai et al., 2021, 3) going through only Stage 2 but not Stage 1, and 4) complete three stage pretraining/fine-tuning as DeepREAL. As shown in Figure 2 and Figure 3 on the evaluation cross three classes (no-binding, agonist, antagonist), the model without any pretraining (i.e., only Stage 3) has the worst performance. Stage 1 or Stage 2 both boosts performance and the complete three-stage pipeline yields the best performance. From the by-class evaluation for antagonist or agonist as shown in Figure 4 and Figure 5, the precision and recall of DeepREAL is mostly higher than other variants in IID, protein OOD, and chemical OOD settings. Furthermore, the training curves of DeepREAL in Figure 4 and Figure 5 converges faster than other variants in most cases. The advantage of pretraining is particularly apparent in chemical distribution shift OOD in the cross-class and the by-class evaluation as shown in Figure 3, Figure 4 and Figure 5. The chemical OOD is a more challenging OOD setting than other settings, where both chemical structure distribution and label ratio balance shift (more details in the following section). DISAE and the only-Stage 3 model have lower Cohen’s kappa, ROC-AUC, MCC than DeepREAL and the Stage2+Stage3 model. The latter two models have relative close performance, suggesting that DTI pretraining plays a more important role than the sequence pretraining in the current training procedure. It may be because the whole Pfam information learned at stage 1 is more difficult to transfer to Stage 3, as supported by the observation shown in Figure 4 and Figure 5. It will be interesting to use other advanced training procedures such as prompting Guo et al., 2020 or design different architectures (e.g., using skip connections) He et al., 2015; Dotskovitsky et al., 2020 etc.).
2.3 DeepREAL is robust in various shifted distribution scenarios

Q2, Q3, Q4 are three typical shifted distribution scenarios in real-world applications, i.e., the OOD generalization challenge. DeepREAL proves robust in each of the settings. It makes DeepREAL applicable to explore dark chemical genomics space.

Q2 focuses on the distribution shift coming from proteins. It is a dominant challenge when applying DeepREAL to a genome-scale given majority of proteins are dark without any receptor activity data. In this setting, 450 proteins and their associated interaction data are split into an OOD train/test sets such that the sequence similarities between proteins in the testing set and those in the training set are less than 10% (Supporting Figure S3). As shown in Figure 2, although the performance drops compared with the easier IIT setting, the ROC-AUC score is still at 0.766, while existing state-of-the-art Random Forest based one-protein-one-model (RF/protein) approach Sakamura et al., 2021 and multi-task neural network model Wang and Zeng, 2013 are totally unable to make reliable predictions in the protein OOD setting.

In a similar fashion, by splitting the data based on the chemical similarity measured by Tanimoto coefficient, DeepREAL is evaluated in the setting of chemical distribution shift to answer Q3. Only Opioid receptors are used in the evaluation because only Opioid receptors have sufficient large numbers of labeled chemicals to generate OOD training/testing data set, as shown in Supporting Figure S2. In addition, we would like to reduce the impact of the OOD from receptors. The advantage of DeepREAL is apparent over other configurations including DISAE, as shown in Figure 3 and Figure 2.3 in the chemical OOD setting. It notes that only around 0.6% of chemicals in the testing set are similar to those in the training set with Tanimoto coefficient larger than 0.6, as shown in Supporting Figure S3.

Although it is expected that a machine learning model performs the best when positive and negative data are balanced, the unseen binding/not-binding cases are imbalanced in reality, which has an estimated ratio of 1:5 Lim et al., 2016. The ratio of 1:5 is based on the estimated value in the published work Lim et al., 2016 when considering the chemical genomics space (millions of chemicals paired with thousands of proteins) as a whole, which is the same scenario as this manuscript. The ratio is lower than the observations from many compound screenings because a large number of potential off-targets are not taken into account in the existing target-based screening. It should be noted that the purpose here is to compare the use of imbalanced test data with the use of balanced ones, which is a common practice in most existing studies. Hence, to answer Q4 about label distribution shift, for all experiments the number of not-binding samples in the test set is about five times as large as that of the agonist/antagonist data while training data are balanced for each class. For a comparison, a balanced test set is also evaluated. In general, DeepREAL evaluated by the balanced test set in the IIT setting, which represents conventional cross-validations, outperforms that evaluated by the imbalanced data. However, in both protein and chemical OOD settings that simulates a real application, DeepREAL evaluated by the imbalanced data performs the best, as shown in Figure 2 and Figure 3. These observations suggest that the cross-validation in an IIT setting is often over-optimistic and DeepREAL is more robust in a realistic application. To see if different imbalanced ratio will affect the result, we performed additional experiments with a ratio of 10:1. As shown in Supporting Material Figure S4, the change of ratio will not change the results significantly.

2.4 DeepREAL significantly outperforms state-of-the-art models

To compare DeepREAL with the leading machine learning model (RF/protein) Sakamura et al., 2021 that can only predict Opioid receptor activities as well as an earlier multi-task neural network model Wang and Zeng, 2013. Only Opioid receptors are used in the comparison due to two reasons. First, the baseline models can be only trained using chemicals as input. Second, only Opioid receptors have sufficient large numbers of labeled chemicals for training the baseline model (Supporting Figure S2). We include other proteins, the baseline model may have significant disadvantages.

Opioid receptor data set is split in two different ways for IIT and OOD experiments as described in the previous section. In both IIT and OOD settings, DeepREAL significantly outperforms the baselines in terms of precision and recall, as shown in Figure 2.3. Furthermore, the performance drop of DeepREAL from the IIT setting to the ODD setting is less significant than that of the baseline. To prove the statistical significance of DeepREAL, performance against the RF/protein baseline, the same training is repeated for five times under opioid context with different random seeds. As shown in the Supporting Figure S5, the p-value of the hypothesis that the two models have the same average ROC-AUC is close to 0.0.

2.5 Application of DeepREAL to cocaine interacting proteins

We performed a screening for G-protein coupled receptors (CPCRs) that interact with cocaine and its analogs using trained DeepREAL model. Cocaine target, cocaine analogs and top ranked predictions could be found in Supporting Information Table S2, S3, and S4. 14 cocaine interacting CPCRs were collected from Fant et al. Fant et al., 2019. We collected 18 cocaine analogs and made predictions on the 14 targets. Among 14 proteins that we tested, cocaine or cocaine analogue was predicted as an agonist for glutamate metabotropic receptor 2 (GRM2) and 5-hydroxytryptamine receptor subtype 6 (5-HT6). As supporting evidences, Yang et al. have showed that GRM2 deletion decreases sensitivity to cocaine reward in rats Yang et al., 2017. 5-HT6 antagonist blocks cocaine-induced DA release and cocaine self-administration, suggesting cocaine probably is a agonist for 5-HT6 Valenti et al., 2013. Our model also predicted cocaine’s antagonist activity against 5-HT2C, delta-Opioid receptor, and Cannabinoid Receptor 2 (CN2R). Injection of the 5-HT2C receptor agonist reduces cocaine self-administration in rats, suggesting cocaine is a potential antagonist for 5-HT2C receptor Fletcher et al., 2004. Similarly, dual kappa-delta Opioid receptor agonist blocks cocaine reward behavior intimating cocaine’s antagonist role for delta Opioid receptor Vlada et al., 2015. Research shows CN2R agonist dose-dependently inhibits cocaine self-administration, thus indicating cocaine negatively regulates CN2R’s activity Xi et al., 2011. Overall, our predictions are largely consistent with existing experimental evidences.

3 Conclusion

This paper proposed a deep learning framework DeepREAL that expands the traditional DTI task to predicting ligand-induced receptor activities of dark proteins and novel chemicals under various OOD settings. DeepREAL has several unique features. First, unlike the existing method that requires training one model for one protein and applying the trained model on the same protein, DeepREAL needs only to train one model to make predictions on any proteins with improved accuracy. Second, DeepREAL has improved generalization power when facing all major types of data distribution shifts during deployment, making it robust in real-world applications. Finally, by utilizing large unlabeled sequence data and rich binary bioassay data, DeepREAL models receptor activities on a multi-scale to alleviate data scarcity problem. Together, DeepREAL significantly outperforms existing algorithms for predicting ligand-induced receptor activities. The novelty of DeepREAL lies in the prediction of receptor activities for dark proteins (stage 3) using pre-trained...
protein sequence embedding (stage 1) and binary DTI embedding (stage 2). The incorporation of stage 1 and 2 pretraining is motivated to achieve the OOD generalization in stage 3. Additionally, the excellent performance of stage 3 is not solely relying on the pretraining of stage 1 and 2. The end-to-end model architecture as illustrated in Supporting Figure S1 is designed to ensure that knowledge transferred over stages won’t be lost and get well utilized. Although DeepREAL was only tested using GPCRs, especially, Opioid receptors due to limited labeled data, it can be extended to other gene families when the ligand-induced receptor activity data are available.

The performance of DeepREAL can be further improved along several directions. For example, unsupervised pretraining of chemical space could improve DeepREAL’s ability to detect novel chemicals.Liu et al., 2021Hu et al., 2019. The sequence embedding method DISAE used in this study still has room for improvement. Incorporating structure information into the protein sequence embedding could help the downstream prediction tasks for ligand binding and receptor activity. Additionally, it may not perform well for small families similar to AlphaFold2/Dumper et al., 2021. It remains an open question to reliably predict the structure and function of dark proteins from a small family. It will also interesting to test other state-of-the-art sequence embedding methods such as ESMRives et al., 2021, ProtBERT/Innagata et al., 2021 and TAPERao et al., 2019. We only predict two classes of receptor activity: agonist versus antagonist. In fact, the receptor activity is more complex than two mutually exclusive classes. There are other subtle activity classes such as partial agonist. A multi-class model could be a more suitable choice and subject to future studies. In practice, detecting if an unseen case is OOD is an important but challenging problem. Few methods have been developed for protein or chemical data for the OOD detection. It is another direction for future works. Furthermore, there are more scenarios of distribution shift worth study such as compounding protein and chemical distribution shifts with various label distribution shifts for stress testing. In addition to the imbalanced ratio of binding/non-binding cases, the ratio of agonist/antagonist could vary a lot for different proteins and there is generally known trend which one is more prevalent. This question remains unanswered, and will be addressed in the future.

4 Methods

4.1 Data

Four data sets were used in this study. Pfam-33.1 Mistry et al., 2020 were used to pretrain protein descriptors. GPCR-ligand binding binary data were obtained from GLASS-2019.2 Chan et al., 2015. Agonist/antagonist data were downloaded from the International Union of Basic and Clinical Pharmacology/British Pharmacological Society (IUPHAR/BPS) Guide to Pharmacology-2020.5. Additional Opioid receptor activity data were from reference Sakamura et al., 2021. The protein descriptor pretraining exactly followed DISAE/Cai et al., 2021. In brief, DISAE built up a distilled triplet sequence dictionary for the whole Pfam proteins based on multiple sequence alignments (MSA). Every input protein was mapped to its distilled triplets representation according to the protein dictionary as illustrated in Figure 1. Chemical-protein pairs with the receptor activity annotation was treated as positive in the binary DTI setting and combined with DISAE for the binary classification pretraining. In terms of pretraining, only stage 1 protein descriptor pretraining was self-supervised as described in the reference Cai et al., 2021. stage 2 uses CLASS data for supervised pretraining. IUPHAR/BPS combined with Sakamura et al., 2021 Opioid data were used in the final stage 3 three-way classification. Detailed data statistics could be found in Table 1.

4.2 State-of-the-art baselines

We compared DeepREAL with Random Forest models for three Opioid receptors/Sakamura et al., 2021 that used PubChem fingerprints1 Bolton et al., 2008 as features. To our knowledge, the RF/protein baseline was the first and only work for the ligand-induced receptor activity prediction. Keeping other hyper-parameters the same as those in Sakamura et al., 2021, the Random Forest depth was tuned to find the best performance model for each Opioid receptor. An example performance curve is shown in Supporting Figure S6. For each experiment, one Random Forest is trained for each Opioid receptor. An average Random Forest test performance was calculated by weighting the sample size of each Opioid receptor. When evaluating the variance of model performance, different random seeds were used.

Another baseline model is similar to restricted Boltzmann machines (RBM) from an earlier work/Wang and Zeng, 2013 which is designed to predict DTI types. We built a multi-task deep learning model that consisted of two layer vanilla MLP/Heaton, 2018 for every single target, i.e. one Opioid receptor, with the same number of hidden units and the same definitions of visible units Heaton, 2018 by optimizing the average cross entropy loss of the model for each target. The constructed multi-task MLP for a multidimensional DTI network was associated with the same parameters. The input feature was also PubChem fingerprints Bolton et al., 2008.

4.3 DeepREAL framework

4.3.1 Architecture

DeepREAL has a novel three-stage framework. There are four major modules in DeepREAL model: protein sequence embedding, chemical structure descriptor, binary interaction learner and multi-class receptor activity classifier as shown in Supporting Figure S1. Under this framework, the state-of-the-art model DISAE/Cai et al., 2021 was employed as the backbone for learning DTI embeddings, which includes ALBERT/Lan et al., 2019 based protein descriptor, and attentive pooling/Santos et al., 2016 based binary interaction learner. Different from DISAE that uses fingerprint for the chemical representation, the chemical descriptor in DeepREAL is state-of-the-art pretrained graph neural network GIN/Xu et al., 2018.

The unique component of multi-class receptor activity classifier includes two sub-modules. A three-way interaction learner uses the same architecture as the binary interaction learner. After concatenating all related embeddings, the concatenated tensor goes through a ResNET/He et al., 2015 layer and MLP/Hastie et al., 2019 transformation to generate the final logit vector used in cross entropy loss calculation Hu et al., 2019.

4.3.2 Information flow of DeepREAL

The knowledge transfer across stages is realized by sharing weights on the first three modules in DeepREAL architecture, i.e., protein descriptor, chemical descriptor and binary interaction learner. Protein descriptor first goes through Stage 1 sequence pretraining in a self-supervised fashion. The pre-trained sequence embeddings are then transferred to Stage 2 binary pretraining. Together with initialized chemical descriptor, a binary interaction learner learns to predict whether or not a protein and a chemical would interact in a supervised learning manner. The learned weights of these three modules are all transferred to Stage 3. In Stage 3, the three modules are first duplicated: one copy has frozen weights whereas the other copy updates its weights for \( n \) epochs with multi-class-learner on DeepREAL receptor activity information in a supervised learning manner, where \( n \) is a hyperparameter as shown in Supporting Information Table S1. In our experiments, we find that a small \( n \) such as 50 would help

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to improve model generalization performance when the training data size was smaller. This phenomenon is due to the fact that a complete model with a large number of trainable parameters is capable of memorizing a small training set, resulting in over-fitting and poor generalization. More frozen weights would limit the over-fitting and put more pressures on the multi-class-learner to learn a robust representation.

As illustrated in Supplemental Figure S1, the protein embedding vector, chemical embedding vector, and binary interaction embedding vector that is the output of binary pretrained module are fine-tuned via a three-way receptor activity learner that also learns a three-way receptor activity embedding. Seven embedding vectors, which include the protein embedding, the chemical embedding, and the binary interaction embedding, both fine-tuned and frozen after the pre-training, along with the three-way receptor activity embedding, are concatenated and fed into a ResNETHe et al., 2015 followed by a MLPHasle et al., 2019 to make the final three-way classification.

The three stage model is designed to train sequentially and separately. Only optimized weights are transferred. The Stage 1 optimization procedure has been described in Cai et al., 2021. Stage 2 and Stage 3 optimization are both driven by a cross-entropy loss in a stochastic manner using AdamKingma and Ba, 2014.

4.3.3 Pretraining implementation and module frozen strategy
A key element of success in multi-stage pretraining is to transfer knowledge. A major challenge in the three-stage pipeline is to prevent the previously learned knowledge from being lost during the weight update in the subsequent stage. DISAE has reported the benefits of a frozen mechanism. This strategy is adopted in DeepREAL Stages 2 and 3 as well. In Stage 2, following the experience of DISAE, part of the transformerVaswani et al., 2017 layers are frozen. In Stage 3, the binary pretrained modules are duplicated to have one copy always frozen and the other copy fine-tuned for only $n$ epochs. Without tuning, $n$ is empirically set to 50 in the Opioid protein focused experiments, while on the complete DeepREAL receptor data set involving 450 proteins, $n$ is set as infinity until the model converges.

4.3.4 Data splitting for training and testing
In terms of data splitting, IID setting splits the data randomly as conventional cross-validations, except for the Opioid context experiments where all three Opioid proteins are ensured to appear in both training and testing data sets. The OOD data split is carried out using a spectral clustering algorithmLuxburg, 2007 based on pair-wise chemical similarity measured by Tanimoto coefficient and sequence similarity measured by sequence identity. The similarity distributions could be found in Supporting Figure S3. In our experiments, the Stage 2 binary training is always carried out with the same data. The pairwise scores in Figure S3 are measured for each pair of a chemical from training and a chemical from test as well as a protein from training and a protein from test. For more than 95% chemicals in the test set, less than 2% chemicals in the training set have Tanimoto coefficient larger than 0.6.

Because we studied several OOD and IID scenarios, in each scenario the number of proteins in the testing set is different.

1. IUPHAR OOD-protein-distribution-shift. The split is made upon protein similarity. 49 out of 450 proteins in the test set. Proteins in the training and testing set have no overlaps. As shown in Figure S3, majority of proteins in the testing set are not similar to those in the training set with the sequence identity less than 10%.

2. IUPHAR IID setting. Data is randomly split. 298 out of 450 proteins are in the test set. 246 out of the 298 proteins in the test set are also in the training set, but there are no overlapped protein-chemical pairs between training and testing set.

3. Opioid OOD-chemical-distribution-shift. The split is made upon pairwise chemical similarity between chemicals in the training set and chemicals in the test set as shown in Supporting Figure S3, where only around 0.6% of chemicals in the testing set are similar to those in the training set with Tanimoto coefficient larger than 0.6. All three Opioid proteins are in the test set.

4. Opioid IID. Data is randomly split. All three Opioid proteins in the test set.

4.4 Ensemble model for novel receptor activity prediction
We build an ensemble of three DeepREAL models independently trained with different random seeds. The ensemble model is used to perform predictions on novel relations. Top predictions are selected by filtering out predictions agreed by all the three models in the ensemble.

5 Data and software availability
All data used are downloaded from PfamMistry et al., 2020, GLASSChan et al., 2015 and IUPHAR/BPS and the state-of-the-art paperSakamuru et al., 2021. Readers are directed to their official website for original data. Code is available on github https://github.com/XieResearchGroup/DeepREAL.

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References


Fig. 1: Illustration of DeepREAL. (A) Given a chemical and a protein sequence as inputs, DeepREAL will predict not only if the chemical is the ligand of the protein but also the ligand-induced receptor activity. (B) DeepREAL is an end-to-end deep learning model trained using three stages of pretraining and fine-tuning. See text for details.

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<td>/</td>
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Table 1. Training, validation, and testing data used in this study
Fig. 2: Performance comparison of DeepREAL with its variants in (A) protein OOD and (B) protein IID settings. The performance is evaluated by multiple gene families in the IUPHAR database.

Fig. 3: Performance comparison of DeepREAL with its variants in (A) chemical OOD and (B) chemical IID settings. The performance is evaluated only by Opioid receptors.
Fig. 4: Training curves of DeepREAL and its variants when measured by the precision for predicting agonists or antagonists. The x-axis is number of training epochs. The y-axis is precision.
Fig. 5: Training curves of DeepREAL and its variants when measured by the recall for predicting agonists or antagonists. The x-axis is number of training epochs. The y-axis is recall.
Fig. 6: Performance comparison of DeepREAL with the Random Forest model (RF/protein) and multi-task MLP in (A) chemical OOD and, (B) chemical IID settings. The performance is evaluated by precision and recall for agonist or antagonist predictions.