1 **Prediction of drug-protein interaction and drug repositioning using machine**

2 **learning model**

3 Yu-Ting Lin¹, Sheh-Yi Sheu^{1,2*}, Chen-Ching Lin^{1*}

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5 ¹Institute of Biomedical Informatics, National Yang-Ming University, Taipei, Taiwan

6 ²Department of Life Science and Institute of Genome Science, National Yang-Ming

7 University, Taipei, Taiwan

8 * Corresponding authors

9

10 **Abstract**

11 **Background**

12 Traditional drug development is time-consuming and expensive, while
 13 computer-aided drug repositioning can improve efficiency and productivity. In this
 14 study, we proposed a machine learning pipeline to predict the binding interaction
 15 between proteins and marketed or studied drugs. We then extended the predicted
 16 interactions to construct a protein network that could be applied to discover the
 17 potentially shared drugs between proteins and thus predict drug repositioning.

1 **Methods**

2 Binding information between proteins and drugs from the Binding Database and the
 3 physicochemical properties of drugs from the ChEMBL database were used to build
 4 the machine learning models, i.e. support vector regression. We further measured
 5 proportionalities between proteins by the predicted binding affinity and introduced
 6 edge betweenness centrality to construct a protein similarity network for drug
 7 repositioning.

8 **Results**

9 As the proof of concept, we demonstrated our machine learning approach is capable
 10 of reflecting the binding strength between drugs and the target protein. When
 11 comparing coefficients of protein models, we found proteins SYUA and TAU that
 12 may share common ligand which were not in our training data. Using the edge
 13 betweenness centrality network based on the prediction proportionality of protein
 14 models, we found a potential target, AK1C2, of aspirin and of which the binding
 15 interaction had been validated.

16 **Conclusions**

17 Our study could not only be applied to drug repositioning by comparing protein
 18 models or searching the protein-protein network, but also to predict the binding

1 strength once the sufficient experimental data was provided to train the protein
2 models.

3 **Keywords:** Drug repositioning, Machine learning, Protein network

4

5 **Background**

6 In pharmacology, drugs can be classified into several classes by their sizes,
7 mechanisms of action, chemical structures or other different criteria. Among these
8 classes, a small molecule drugs (SMD) often refers to an organic compound with low
9 molecular weight (< 900 dalton) and small size. Compared to biological drugs, SMDs
10 have simple and well-defined structures, leading to easier synthesis and a more
11 predictable chemical process in manufacturing and characterizing. In general, the
12 development of one SMD is mainly processed via 5 stages: I. *Discovery and*
13 *Development*, II. *Preclinical Research*, III. *Clinical Research*, IV. *FDA Review*, and V.
14 *FDA Post-Market Safety Monitoring*, to make sure that the new pharmaceutical
15 compounds are safe to be distributed to the market [1, 2]. Among the cycle of drug
16 development, the first step, drug discovery, is one of those that cost the most, up to
17 674 US\$ millions per one approved drug and taking 2~5 years in average [3-5]. To
18 boost the process, novel techniques were proposed in recent years, like combinatorial
19 chemistry methods for compound formulation as well as using virtual screening and

1 high-throughput screening to filter out candidate drugs. By using a computational,
2 data-based, or robotic method, the accuracy and efficiency of drug discovery were
3 highly improved. Also, drug repositioning plays an important role in accelerating drug
4 development as well.

5 Drug repositioning (a.k.a. drug repurposing) has increasingly become an essential
6 strategy in drug development by reinvestigating existing de-risked and approved
7 drugs for new therapeutic purposes [6]. Despite some remaining technological and
8 regulatory challenges needed to be solved, this novel strategy is more efficient,
9 economical, and riskless. On the other hand, with a great amount of biochemical,
10 pharmacological, and clinic data collected in these decades, there have been many
11 emerging techniques like machine learning and network analysis that can take
12 advantage of big data and promote efficiency and productivity [7].

13 So far, studies using machine learning approaches for drug repositioning have
14 become more and more popular [8-10]. Napolitano F, et al. (*J Cheminform*, 5, 30,
15 2013) proposed a method based on an SVM (support vector machine) classifier to
16 predict the corresponding disease classes of drugs [11]. Kim E, et al. (*BMC*
17 *Bioinformatics*, 20, 247, 2019) constructed models using multiple classification
18 algorithms, including logistic regression, random forest, and SVM to predict potential
19 indications for existing drugs [12]. The method developed by Zhanchao Li (*Anal.*

1 *Methods*, 10, 4152-4161, 2018) uses the random forest algorithm to predict the
2 binding affinity between a compound and a protein directly. Based on the graph
3 theory, the model uses compound-compound similarity, protein-protein interactions,
4 and compound-protein interaction to characterize the binding interaction and may be
5 applied to high-throughput virtual screening [13].

6 In this study, we employed the support vector regression (SVR) algorithm to build
7 models to predict the binding interaction between proteins and drugs. The inhibition
8 constant (K_i), which represented the binding strength, was used as label; and
9 structural fingerprint format and physicochemical properties of drugs were used as
10 features. Consequently, we selected 556 protein models to forecast the binding
11 strength with 86,475 drugs. Additionally, we adapt the similarity of predicted binding
12 strength to drugs between proteins to construct a protein similarity network.
13 Furthermore, we proposed an auxiliary network constructed from edge betweenness
14 centralities (eBC) to discover the potential drug repositioning between proteins. With
15 the aid of the network, we found a potential target protein AK1C2 of aspirin by its
16 known target PGH1 and the binding interaction had been reported, providing a
17 successful application of our method for drug repositioning. Briefly, our method can
18 not only be applied to drug repositioning by comparing protein models or searching

1 the protein-protein network, but also by predicting the binding strength directly once
2 the experimental data are sufficient to train the protein models.

3 **Methods**

4 **Information of proteins and drugs**

5 To predict the strengths of unknown binding interactions of proteins and drugs, we
6 needed experimental data of known protein-drug interactions train the machine
7 learning model. In this study, the protein-drug interaction data were downloaded from
8 the Binding Database [14-18] and interactions with non-human proteins were
9 discarded. Next, the drug compounds are provided with the structural information,
10 PubChem CID, InChIKey, and the binding strength to the target protein, which is
11 inhibition constant in this study, were recognized as informative and kept. For
12 proteins, only human proteins with specific UniProt entry names were adopted.
13 Among the information, the binding strength is the label that our machine learning
14 model attempts to predict; and structural information is one part of the feature in the
15 model. Noted that in our study, only drug information was used as features to describe
16 the binding interactions in each protein model. The circumstances of multiple binding
17 sites of a protein were not considered and only the data with the highest value of
18 binding strength was kept if there were more than one data of a drug-protein pair.

1 The structures of drugs were described as “Molecular ACCess System (MACCS)
2 keys” [19-21] that consists of a list of binary values (0 or 1) and contains 166 key
3 descriptors to encode a structure. The MACCS keys of drugs were used as a 166-bit
4 feature to train the machine learning models.

5 On the other hand, since the property data describing physicochemical
6 characteristics of a drug could affect the binding between drug and target protein, we
7 further add them as features in our model. The physicochemical properties of drug
8 compounds were obtained from the ChEMBL database. We used the Python library
9 “chembl_webresource_client”, which is officially developed and supported by the
10 ChEMBL group and selected 17 properties that might have relevance with binding
11 and thus help in prediction. The properties are introduced following their names used
12 in the library (Table S1). Finally, the 17 physicochemical properties were combined
13 with the 166-bit structural fingerprint to form 183 features that were used in our
14 machine learning model.

15 **Binding strength of drug-protein interactions**

16 In this study, we used $-\log K_i$ as label to represent the binding strength of a
17 drug-protein interaction and to train the protein models. In fact, the Gibbs Free
18 Energy (ΔG^0) is also frequently used to represent the binding affinity of a drug and
19 target. From the aspect of thermodynamics, the lower the ΔG^0 is, the stronger the

1 interaction is. And ΔG^0 can be calculated by an equilibrium constant (K_{eq}) as
 2 $\Delta G^0 = -RT \ln K_{eq}$, where R is the ideal gas constant that equals $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$
 3 and T is the temperature on the Kelvin scale [22]. The equilibrium constant is the
 4 dissociation constant in this case, then the ΔG^0 can be further represented by K_i as
 5 $\Delta G^0 = -RT \ln \frac{1}{K_i}$. Because R and T are both constants in the equilibrium, we can
 6 infer that ΔG^0 is positively proportional to $\log K_i$ by multiplying $\ln K_i$ by a constant
 7 $\log e$. For the reasons mentioned above, we chose $\log K_i$ to represent the strength of
 8 the binding of a drug-protein interaction.

9 **Construction of protein models**

10 In this study, we aim to develop a machine learning model to predict the binding
 11 affinity of a drug to a specific protein. The machine learning algorithm we chose here
 12 is support vector regression (SVR), which is a regression version of support vector
 13 machine (SVM) and works by finding a hyperplane that can minimize the distance
 14 from the hyperplane to the instance and cover as most data points as possible in the
 15 same time [23]. Because the model of SVM or SVR can be recognized as a weighted
 16 sum of the support vectors, it has advantages of reduced computational time and
 17 storage requirements over other frameworks. And the principle gives SVM or SVR
 18 good generalization capability and better robustness over those algorithms that
 19 involve randomness elements.

1 To train our prediction model, we obtained 152,112 binding information among
2 1,042 proteins and 86,475 drugs from the BindingDB (Dataset S1) [14-18]. The drug
3 in each data was described by 183 features which consisted of a 166-bit MACCS
4 structure fingerprint [19-21] and 17 physicochemical properties (Table S1) from the
5 ChEMBL database (Dataset S2) [24, 25]. Here we introduced inhibition constant (K_i)
6 to represent the binding strength of a drug with its target protein. K_i is defined as
7 $K_i = \frac{[E][I]}{[EI]}$ where [E], [I], and [EI] are the equilibrium concentrations of enzyme,
8 inhibitor, and the complex [26]. The smaller the K_i value, the greater the binding
9 strength of the inhibitor with its target, and the better the potency of the drug in most
10 cases. Since the K_i value can quantitatively reflect how tightly a compound binds to a
11 particular protein, we used it to represent the binding strength of a compound-protein
12 interaction. In our data, however, the experimental K_i values show an exponential
13 distribution and many of the experimental K_i values are larger than 1,000 while some
14 of the others are less than one. Given that many regression algorithms are based on
15 the concept of least squares method to approximate data, the large difference in scale
16 between observations leads to poor fitting since the residues on the larger scale weigh
17 moreover those on a smaller scale. Therefore, we transformed the experimental K_i
18 values into logarithmic ones, to offer the machine learning model a dataset with a
19 linear distribution. Furthermore, to make the study results easier to be comprehended,

1 we used $-\log K_i$ as the indicator of binding affinity and the label of our machine
2 learning model, while the larger the value is, the stronger the compound-protein
3 interaction is.

4 Before model training, each property feature among samples were standardized
5 by computing the standard score z as $z = (x - \mu) / \sigma$, where μ is the mean and σ is
6 the standard deviation of the feature throughout all samples. We then built a
7 prediction model for each protein using only the dataset targeting it. The model
8 trainings were all default settings except the parameter “kernel” was specified to be
9 “linear”. When training the SVR model, we randomly split the dataset into two groups:
10 70% of the dataset as the training set to build the model and evaluate the prediction
11 accuracy, and the other 30% as the testing set for validation to assess how the model
12 would generalize to an independent dataset. Then the models applied for further
13 analysis would be trained by their complete datasets.

14 The evaluation we used here to see how well a model could predict was the
15 Spearman correlation coefficient (SCC, ρ), which is a nonparametric measure of rank
16 correlation to assess the monotonic relationships [27-29]. When comparing the
17 accuracies among models, we introduced the Fisher transformation to take the data
18 number into account [30]. The transformation of the sample SCC value r can be
19 computed by

$$F(r) = \frac{1}{2} \ln \left(\frac{1+r}{1-r} \right).$$

When given the sample size n , the z -score of r is

$$z = \sqrt{\frac{n-3}{1.6}} F(r),$$

which approximately follows a standard normal distribution under the null hypothesis of statistical independence ($\rho = 0$).

Construction of the protein network for drug repositioning

Having the predictions of binding strength, the protein similarity was calculated by proportionality [31-33]. However, since the protein similarity only considers the direct association between them, we further constructed an auxiliary network which was based on proportionalities and was capable of detecting the indirect similarity between proteins.

To keep the highly confident protein pairs, we first removed the protein pairs with low proportionality, which is the majority of the network, and applied the concept of the shortest paths in the network to consider the indirect correlations since the two adjacency proteins might not have the strongest correlation when computing direct similarity.

The concept of the shortest path was applied in our study by betweenness centrality, which is used to evaluate the importance of nodes of edges in a network. If a network is weighted, the shortest path is the one having the maximal sum of the

1 weights of the edges. The edge betweenness centrality (eBC) of an edge is defined by
 2 the number of these shortest paths that pass through this edge and the eBC of an edge
 3 v can be computed as

$$g(v) = \sum_{s \neq v \neq t} \frac{\sigma_{st}(v)}{\sigma_{st}},$$

4 where σ_{st} is the sum of the shortest path from node s to node t , and $\sigma_{st}(v)$ is the
 5 number of the paths that pass through v [34].

6 For our original network of proportionality, we calculated the eBC of every edge
 7 in it and removed those whose eBC was 0. Then each remained edge was assigned a
 8 new weight by its eBC value for next eBC calculation. The cycle of eBC calculation,
 9 removing edges, and assignment of new weights was repeated until convergence
 10 (Figure S1A). Here we used the mean-square error (MSE) between the edge weights
 11 and the eBC values in a cycle to tell whether the calculation was converged. The
 12 number of remained edges maintained the same after the 15th cycle and so did the
 13 MSE after the 18th (Figure S1B). We then used the eBC values calculated in the 18th
 14 cycle to build an eBC-based protein-protein network for further analysis.

15 **Results**

16 **Proof of concept for the machine learning model**

17 To determine the capability of our machine learning pipeline in predicting the binding
 18 affinity of a drug to a specific protein, we first used the models trained from AA2AR,

1 DHI1, and AL5AP separately as a proof of concept. First, we partitioned our dataset
 2 into training and testing sets to perform cross-validation for these three proteins. We
 3 observed that the experimental $-\log K_i$ values were strongly and positively correlated
 4 with the predicted $-\log K_i$ values from training and testing sets (Table 1 and Figure 1a).
 5 These results implicated that this approach might be capable of reflecting the binding
 6 strength of a drug to target protein well to some extent. However, the process of
 7 random sampling for cross-validation makes deciding the best model uncertain.
 8 Additionally, to get the best performance when given an unknown drug, the complete
 9 dataset targeting a specific protein should be used to train the model. Hence the
 10 accuracy and the risk of overfitting using the complete dataset to train and test the
 11 model were examined (Figure 1a). The SCC values of complete datasets in the three
 12 models were close to the distributions of the training sets as well as testing sets (Table
 13 1), demonstrating the low risk of overfitting from complete dataset. Though the SCC
 14 values themselves may be affected by the number of drugs, leading to lower
 15 accuracies of the complete datasets than the training sets, the models built by
 16 complete datasets were still representative for predictions. Thus, the models in the
 17 following study were trained by their own complete dataset for best predictions.

18 **Table 1. Performance of three models: AA2AR, DHI1, and AL5AP.**

Model	AA2AR		DHI1		AL5AP	
	Training	Testing	Training	Testing	Training	Testing

Number of Drugs	3452		259		1411	
Avg. SCC	0.72	0.68	0.91	0.68	0.7	0.62
Range of SCC	0.7~0.74	0.63~0.73	0.87~0.95	0.42~0.84	0.67~0.73	0.53~0.71
SCC of complete dataset	0.71		0.89		0.69	

1

2 We further examined the ability of a protein model to select the drugs that would

3 have binding interaction with the corresponding protein from all the drugs in the

4 dataset. For example, the $-\log K_i$ values of all 86,475 drugs predicted from the AA2AR

5 model were compared with that of AA2AR experimentally-validated drugs. We found

6 that the $-\log K_i$ values distribution of experimentally validated drugs were significantly

7 higher than those of other drugs (Figure 1b). Additionally, the experimentally

8 validated drugs were observed to be significantly enriched in the drugs with higher

9 predicted $-\log K_i$, and the amount decreased over the rankings. (Figure 1b). This

10 supported that our model could not only pick out the drugs that were already known

11 to have binding interaction with the modeled protein but also implicate the

12 unvalidated drugs in the top rankings may have potential binding ability to the

13 modeled protein. To further demonstrate that the models could predict the drugs

14 strongly binding with the modeled protein, we investigated the drugs with high

15 predicted and experimental $\log K_i$ to AA2AR. The compound of

16 3-[2-[4-[2-Fluoro-4-(2-methoxyethoxy)phenyl]piperazin-1-yl]ethyl]-8-(furan-2-yl)-[1,

17 2,4]triazolo[5,1-f]purin-5-amine is predicted with the secondly highest $-\log K_i$ value of

0.094 among the drugs targeting AA2AR and possessed the 99th high experimental $-\log K_i$ value of 0.097 in the training set. The experimental k_i value of the compound binding to AA2AR is 0.8 nM, making the compound a promising antagonist to AA2AR. The possible application of the drug could prevent motor disturbances caused by defects related to dopamine D2 receptors and further serve as an anti-symptomatic drug for the treatment of Parkinson's disease [35]. Briefly, using AA2AR, DHI1, and AL5AP models as exemplifying proof of concept showed that our method based on the machine learning approach could be used to predict the relative binding strength between a drug and a protein, which could be applied to the screening of candidate drugs in drug development.

Performance of the machine learning model

After demonstration of the proof of concept for our machine learning pipeline, we then attempted to construct the prediction models for all the proteins in the database. However, the machine learning approaches were known to be affected by the sample size - number of experimentally validated drugs in this study. We also observed that, compared to AA2AR and AL5AP model, DHI1 model, which possessed fewer experimentally validated drugs, showed larger difference of SCC between training and testing set (Figure S2). Therefore, we verified the effect of the training data size on the performances of the prediction models. Among the total 1,042 proteins from

1 the BindingDB, over 46% of them have less than 10 data of binding information with
 2 drugs and only 21% with more than 100 data (Figure 2a). Furthermore, we found that
 3 as the number of drugs increased, the SCC z-score values of model predictions
 4 became higher, suggesting better accuracies (Figure 2b). Also, the more
 5 experimentally-validated drug data a protein model was built by, the smaller the
 6 standard deviation of the SCC distribution (Figure 2c) and the more unapparent the
 7 SCC difference between the model trained by the complete dataset and by the training
 8 set (70% of complete dataset) were observed (Figure 2d). These results also indicated
 9 that the model trained for the protein with more experimentally validated drugs could
 10 be more stable. Accordingly, to prevent models from overfitting and inaccuracies
 11 caused by lacking training data, 556 proteins (53% of all) with more than 10 drug data
 12 were retained for the following analysis. Among 556 proteins, 513 (92.26%) protein
 13 models are provided with SCC z-score greater than two, suggesting the high accuracy
 14 of the machine learning pipeline in predicting relative $-\log K_i$ between drugs and
 15 proteins.

16 Next, we testified the model specificities by using the datasets from the other
 17 proteins. For example, to test the AA2AR model, we used it to predict the $-\log K_i$ of
 18 data originally targeting DHI1 and AL5AP. Then, for DHI1 and AL5AP, we compared
 19 their $-\log K_i$ predicted by AA2AR model to their experimental $-\log K_i$ separately. As

1 expected, the SCC between experimental $-\log K_i$ targeting DHI1 and predicted $-\log K_i$
2 from AA2AR model was -0.21; and the SCC between experimental $-\log K_i$ targeting
3 AL5AP and predicted $-\log K_i$ from AA2AR model was 0.09 (Figure S3). The two
4 results suggested that the AA2AR model could not be used to predict the $-\log K_i$ of
5 drugs binding to DHI1, neither AL5AP. Same evaluation was performed on DHI1 and
6 AL5AP models and it was alluded that a protein model could only be applied to
7 predict the drugs targeting the protein itself and showing the specificity of the
8 prediction model (Figure S3). The investigation of all the models was further
9 evaluated by the distribution of the average z-score. For all 556 proteins, every model
10 was used to predict the $-\log K_i$ of other 555 sets of drugs and their target proteins, and
11 the average z-score value was recorded (Figure 2e). Notably, only 1.6% proteins are
12 provided with average z-score greater than two, showing that most of the models were
13 unsuitable to predict the binding interaction targeting proteins other than themselves
14 and assuring the overall specificities of models.

15 Nevertheless, based on the reason, the specificities of models indicated that if a
16 model could predict the drugs of another protein well, there might be some
17 similarities or shared characteristics between the two proteins that could be applied to
18 drug repositioning. For example, the model of protein DRD2 was trained by 5,155
19 drug data and had a z-score of 20 when predicting the 844 drugs binding to their

1 experimental target protein BCL2. The two proteins shared significantly enriched GO
2 biological process (p-value < 0.0001, Fisher's exact test), showing their functional
3 similarity. DRD2 is dopamine D2 receptor whose activity is mediated by G proteins
4 and is the main receptor for most antipsychotic drugs [36]. BCL2 is apoptosis
5 regulator (B-cell lymphoma 2) which can suppress or induce apoptosis and regulate
6 cell death by controlling the mitochondrial membrane permeability [37, 38]. The
7 relevance of the two proteins in several signaling pathways has been studied. In
8 non-small cell lung cancer, it was found that the upregulation of BCL2 is related to
9 the knockdown of DRD2 and the overexpression of the DRD2 gene may decrease the
10 mRNA level of BCL2 [39]. In another research on Parkinson's disease, it was found
11 that the inhibition of hsa-miR-200a can upregulate BCL2 and DRD2 via the
12 cAMP/PKA signaling pathway and suppress apoptosis in striatal neuron cells [40].
13 Though no research reported the shared drugs of these proteins, we still demonstrated
14 that using model specificity could help find proteins with similar biological roles and
15 the relevance of them may provide information for medication.

16 **Model comparison and drug repositioning**

17 We have demonstrated that the SVR models could be able to predict the
18 relative $-\log K_i$ value between a drug and a target protein. Therefore, our approach
19 could be a feasible application in compound screening during drug development,

1 especially for repositioning of the approved drugs that are provided with detail and
2 complete experimental information. For example, two proteins with similar models
3 after training might possess similar drug binding characteristics. Accordingly, their
4 models might be able to predict promising drugs as a potential treatment for each
5 other, a.k.a. drug repositioning. We then calculated the SCC of coefficients between
6 models to assess the similarity of drug-binding profiles between proteins. That is, two
7 proteins with higher SCC are more similar to each other on drug-binding
8 characteristics. Accordingly, for each model pair, we inspected the association
9 between the SCC of model coefficients and the average drug number (Figure 3). The
10 results showed that when the models were trained by few drugs, their model
11 similarities varied from 0.89 to -0.56 and when trained by more drugs, the model
12 similarities became more convergent and lied mostly between ± 0.2 . The p -values
13 were examined and the smallest positive SCC value with significance (p value < 0.05)
14 was 0.15 (the green line in Figure 3). The proteins of the model pairs above the
15 significance line could be worth to be investigated for drug repositioning. Among all
16 the protein pairs, three of them are with more than 3,000 drugs in average and SCC
17 higher than 0.3. In two of the pairs, CAH1-CAH2 and DRD2-DRD3 were from the
18 same family. Since proteins in a family typically have similar structures, functions,
19 and sequence similarity, it was reasonable for their prediction models with similar

1 feature weight ranking, that is, with high SCC. This also demonstrated that our
 2 method could recognize the proteins with some shared characteristics. However, for
 3 the proteins in the same family, most of the experimented drugs were the same,
 4 limiting the efficacy of repositioning. Thus, we focused on another protein pair,
 5 5HT2A-DRD3. Protein 5HT2A is a G-protein coupled receptor for
 6 5-hydroxytryptamine (serotonin) and can affect neural activity, perception, cognition,
 7 and mood. It also plays roles in the regulation of behavior, intestinal smooth muscle
 8 contraction, and arterial vasoconstriction [41, 42]. DRD3 is a dopamine receptor
 9 whose activity is mediated by G proteins which inhibit adenylyl cyclase and can
 10 promote cell proliferation [43]. Among 2,683 drugs of 5HT2A and 3,535 drugs of
 11 DRD3, 914 shared drugs are significantly over-represented (p -value < 0.0001 ,
 12 Fisher's exact test), suggesting that they did share binding interaction that had been
 13 under study.

14 However, for the models trained by few drugs, their comparisons may not be
 15 convincing enough with a wide range of model coefficients similarities since it was
 16 demonstrated that the models performed better with more drugs. Thus, once there
 17 were models that were trained by enough data and had high correlations, the two
 18 proteins may have alike binding interactions. Another problem was that the difference
 19 in the sample size of models may lead to deviations when comparing the coefficients

1 directly, making it inappropriate to build a network by model similarities. Therefore,
 2 we proposed the construction of a protein-protein network based on prediction
 3 similarity between all protein models to reduce the data amount effect on a single
 4 model and provide insights on the mutual connection of proteins.

5 **Drug repositioning using the eBC network**

6 To apply the protein models to drug repositioning from an integral aspect, we
 7 constructed protein similarity networks guided by the SVR model (Figure 4a and 4b).
 8 In the network, edges linking proteins are weighted by the proportionality calculated
 9 from the predicted $-\log Ki$. To note, the distances between proteins in the network were
 10 assigned by $1 - \text{proportionality}$. In other words, edges with higher proportionality, i.e.
 11 more similar, were assigned by shorter distance. Next, we used the edge betweenness
 12 centrality (eBC) to identify the shortest (highest similarity) paths between proteins.
 13 Accordingly, the indirect association between proteins, that is, the paths with highest
 14 similarity included more than one proteins, could be discovered. Interestingly, the
 15 average GO term similarity of protein pairs with a two-step distance was the highest
 16 (Figure 4c), meaning that when computing similarities via one intermediate protein
 17 allows the eBC network to represent the correlation of the roles that the proteins play
 18 in biological processes. Hence it may be feasible for drug repositioning to consider

1 the proteins' second neighbors in the eBC network if they would have shared drugs
2 and binding interactions.

3 To determine the reliability of our eBC network for drug repositioning, we chose
4 aspirin (acetylsalicylic acid) as an example to find its potential target protein. Aspirin
5 is a drug widely used to reduce pain, fever, or inflammation, and also applied to
6 prevent strokes and blood clots. One of the known target proteins of aspirin is
7 cyclooxygenase-1 (COX-1), which will be irreversibly inhibited by the acetylation of
8 a serine residue in the active site [44-46]. Basing on COX-1, we then tried to find
9 proteins in the eBC network that may also be targets of aspirin. Among the proteins
10 that were in two-step distance from COX-1, AK1C2 had the highest 2-step GO term
11 similarity with a value of 0.67. AK1C2 is aldo-keto reductase family 1 member C2
12 that is encoded by gene *AKR1C2*. The enzyme catalyzes the inactivation of
13 5-alpha-dihydrotestosterone (5-alpha-DHT) to
14 5-alpha-androstane-3-alpha,17-beta-diol (3-alpha-diol) [47, 48]. The selective loss of
15 AK1C2 may have the relevance of breast and prostate cancers [49, 50] and the
16 overexpression of it may be a high-risk factor in bladder cancer [51]. In our dataset
17 used to train the AK1C2 model, none of the data was about the binding of aspirin to
18 AK1C2, leaving aside the possibility that the network had already had the information
19 of this binding interaction. In a paper studying the inhibition of AK1C2 by

1 nonsteroidal anti-inflammatory drug (NSAID) analogs proposed in 2009 by Michael
2 C. Byrns et al., it was reported that IC₅₀ value of inhibition of AK1C2 by aspirin was
3 16 μM. Also according to the study, the concentrations of aspirin and its deacetylated
4 product salicylic acid needed for inhibition were lower than those of other NSAIDs
5 [52].

6 The GO term similarity of biological process between COX-1 (PGH1) and
7 AK1C2 in our network was calculated via EST2, a cocaine esterase. Most of their
8 shared GO biological process terms were related to the metabolic process for
9 substances including lipid, organic acid, carboxylic acid, and others. The other terms
10 were mainly related to cellular responses to stimulus. Though without support from
11 known experimental information, it might be possible that there were some
12 correlations between the shared drug and common GO biological process terms of the
13 two proteins, which was worthy of further studying.

14 **Discussion**

15 We have proposed a machine learning pipeline to discover the potential drug
16 positioning for proteins. To further understand which structure characteristics of drugs
17 affecting the binding with proteins, we analyzed the feature importance of the learning
18 functions. Here we chose three models with the highest SCC z-score values of
19 predictions and inspected the absolute values of weights of their physicochemical

1 property features. The protein model with the highest z -score of 52.2 among all the
2 models was trained from FA10 by 3,158 binding data. Among 17 physicochemical
3 property features in the FA10 model, the absolute values of their coefficients ranged
4 from 0.05 to 0.89. The feature with the largest absolute weight of -0.89 was the
5 number of non-hydrogen atoms in the molecule, accounted for 10.5% of the 17
6 features. The one with the second-high absolute value of weight was the polar surface
7 area (0.84, 9.9% of all); the third feature was the molecular weight of the parent form
8 of the molecule (0.83, 9.9% of all). The protein model with the second-high
9 prediction z -score of 50.89 was AA2AR trained by 3,452 data. The absolute values of
10 its features' coefficients ranged from 0.01 to 1.09. The three top features in
11 descending order of their absolute values of weights were the number of
12 non-hydrogen atoms in the molecule (1.09, 22.3% of all), the count of hydrogens
13 attached to nitrogen or oxygen atoms (0.79, 16.2% of all), and the number of
14 hydrogen bond donors (0.48, 9.9% of all). The third-high z -score of 47.63 belonged to
15 the TRY1 model trained by 986 data. The absolute values of 17 feature coefficients
16 ranged from 0.01 to 1.34. The three top features in descending order of their absolute
17 values of weights were the count of nitrogen and oxygen atoms in the molecule (-1.34,
18 23.1% of all), the polar surface area (1.01, 17.4% of all), and the count of hydrogens
19 attached to nitrogen or oxygen atoms (-0.518, 8.9% of all). The last model examined

1 was DRD3, which had the forth-high z-score value of 47.43 and was trained by 3,535
2 data. Its absolute values of 17 feature coefficients ranged from 0.01 to 1.25. The three
3 top features in descending order of their absolute values of weights were the number
4 of non-hydrogen atoms in the molecule (-1.25, 23.4% of all), the value of logP (0.73,
5 13.8% of all), and the polar surface area (0.69, 13% of all).

6 Among the four models, three of them had polar surface area (PSA) as an
7 important feature, which is defined as the surface sum over all polar atoms or
8 molecules [53]. In general, bindings between proteins and drugs will be affected by
9 non-bonded interactions, which can be further divided into electrostatic interactions
10 and Van der Waals interactions. While many of the binding interactions depend a lot
11 on the formation of hydrogen bonds and salt bridges, the ligands with more PSA
12 might bring about stronger electrostatic interactions. Also, the PSA property is related
13 to the solvation of a molecule and will affect the inhibition ability of a drug to its
14 target protein. Therefore, PSA played an important role in binding strength prediction
15 and weighed higher in our models [54].

16 The other features with high weights were also associated with hydrogen atoms
17 and hydrogen bonds, including the number of hydrogen bond donors (HBD), the
18 count of nitrogen and oxygen atoms (HBA Lipinski), count of hydrogens attached to
19 nitrogen or oxygen atoms (HBD Lipinski), and the number of non-hydrogen atoms in

1 the molecule. Unlike PSA, the weights, or coefficients, of these features differ in sign.
 2 This is probably because that different target proteins may serve as donors or
 3 acceptors of hydrogen bonds, while the number of donors/acceptors of drugs and the
 4 binding sites of proteins also contribute to the interaction, leading to diverse
 5 prediction pattern in different protein models.

6 Next, we tried to apply the similarity of feature coefficients between protein
 7 models to drug repositioning. Among all 154,290 protein pairs, there were 37 pairs
 8 with SCC values of coefficients higher than 0.5 among, and of which each of the 35
 9 pairs was from the same protein family. Since proteins in a family typically have
 10 similar structures, functions, and sequence similarity, it was reasonable for their
 11 prediction models with similar feature weight ranking, that is, with higher SCC. Also,
 12 for the proteins in the same family, most of the experimented drugs were the same,
 13 limiting the efficacy of repositioning.

14 Thus, to further discover the capability of identifying the drug repositioning
 15 between proteins from different family, we tried to study the other two protein pairs,
 16 SYUA-TAU, and CY24B-WEE1. Since no research of common ligands to these two
 17 proteins was found, this makes it difficult to demonstrate the association between
 18 CYBB and WEE1. Additionally, the numbers of drug data used to train the models are
 19 relatively small, which were 19 and 14 for CY24B and WEE1, respectively. The size

1 of training sets might not be enough for models to approximate precise functions for
 2 prediction, and lead to bias when comparing model coefficients. Consequently, we
 3 focused on the protein pair, SYUA-TAU. The coefficients of the models of SYUA and
 4 TAU had the second-high correlation among all the protein pairs, with an SCC value
 5 of 0.86. SYUA is a protein called alpha-synuclein encoded by the *SNCA* gene. It
 6 mainly locates in the brain other than other tissues and is critical for brain function
 7 [55]. And TAU is a protein named microtubule-associated protein tau, which is
 8 encoded by gene *MAPT* [56, 57]. TAU can stabilize microtubules and is abundant in
 9 neurons of the central nervous system. Both proteins are associated with pathologies
 10 and dementias of the nervous system like Parkinson's disease and Alzheimer's disease,
 11 in which abnormal aggregations into amyloid of these proteins are often observed. In
 12 these disorders, the alpha-synuclein protein and Tau protein form insoluble fibrils that
 13 are characterized by Lewy bodies and neurofibrillary tangles respectively [58].

14 The SYUA model and TAU model were built by 45 and 46 drug data separately,
 15 while the 45 drugs were the same and the TAU model had one more drug, FDDNP, in
 16 the training dataset. FDDNP
 17 (2-(1-{6-[(2-[fluorine-18]fluoroethyl)(methyl)amino]-2-naphthyl}-ethylidene)malono
 18 nitril) is a positron emission tomography (PET) molecular imaging probe used to
 19 visualize Alzheimer's disease pathology and its ability to detect amyloid in the brain is

1 already proved. Due to the model similarity between the two proteins, FDDNP might
 2 also have binding interaction with SYUA. Additionally, it has been reported that
 3 FDDNP has a binding interaction with alpha-synuclein filaments *in vitro* and the K_i
 4 value is 210 nM, while in our study the K_i value of FDDNP binding to TAU was 256
 5 nM [59]. The two close experimental K_i values suggested that by comparing model
 6 coefficients, we could find the proteins that may have similar binding patterns and
 7 even share common ligands. However, FDDNP is majorly used as radiotracers of PET
 8 for clinical diagnosis for Alzheimer's disease and seldom serves as direct medication
 9 for treatments. This case demonstrated a promising application in drug repositioning,
 10 though it is not the direct usage in treatments.

11 Our study so far focused on building single model for each protein and used only
 12 drug structures and physicochemical properties as features. In the future, an advanced
 13 goal is to incorporate features of protein and drug to construct a general model that
 14 can predict the binding strength once given a protein-drug pair. Unlike the single
 15 models that were restricted to the amount of experimental data of proteins, the general
 16 model takes protein features like structures into account and is trained by the binding
 17 information targeting all proteins, making it more feasible for prediction of rare
 18 protein-drug instances.

1 **Conclusions**

2 In this study, we applied a machine learning approach to predict the drug-protein
3 interaction and extended the result to construct a protein-protein network for drug
4 repositioning. We demonstrated that the models trained by more drug data had better
5 performance and less risk of overfitting when comparing the SCC values between
6 training and testing results. We further showed that our protein models were able to
7 recognize their own drugs from all the compounds in the dataset, which suggested that
8 the models could be applied for screening of candidate drugs. Also, by comparing
9 model coefficients of physicochemical properties, protein pair SYUA-TAU was found
10 to have alike biological role on GO term annotation and similar binding interactions
11 with shared drugs. When using protein eBC network based on proportionality for drug
12 repositioning, we found a potential target protein AK1C2 of aspirin by investigating
13 the two-step neighbors around its known target PGH1. Briefly, our method can not
14 only be applied to drug repositioning by comparing protein models or searching the
15 protein-protein network, but also by predicting the binding strength directly once the
16 experimental data are sufficient to train the protein models. When given a drug with
17 structure and property information, we can get its binding strengths targeting different
18 proteins and find the one with the highest value as a new medication. Also given a
19 specific protein, we can use its model to predict all the drugs that have experimental

1 data and find those with high returned values for alternative medication. As the
 2 accumulation of experimental data and clinical research, we believe that the problem
 3 raised by data size will be solved soon in the future and by using our method, the
 4 efficiency and cost of drug development can be greatly improved.

5

6 **List of abbreviations**

7 **SMD:** small molecule drugs

8 **FDA:** Food and Drug Administration

9 **SVM:** support vector machine

10 **SVR:** support vector regression

11 **SCC:** Spearman correlation coefficient

12 **eBC:** edge betweenness centrality

13 **MSE:** mean-square error

14

15 **Declarations**

16 ● **Ethics approval and consent to participate**

17 Not applicable.

1 ● **Consent for publication**

2 Not applicable.

3 ● **Availability of data and materials**

4 The datasets used and analyzed in this study are available in the Binding DB website
5 (<https://www.bindingdb.org>); the ChEMBL repository (<https://www.ebi.ac.uk/chembl>);
6 and the Gene Ontology resource (<http://geneontology.org/>).

7 ● **Competing interests**

8 The authors declare that they have no competing interests

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14 ● **Authors' contributions**

15 SYS and CCL directed this study. CCL conceived and designed this study. YTL
16 implemented the computational method and carried out the analysis. YTL drafted the

1 manuscript. SYS and CCL revised the manuscript. All the authors read and approved
2 the final manuscript.

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7

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7 **Figure Legends**

9 **Figure 1. Performance of the prediction model from AA2AR, DHI1, and AL5AP**

10 **protein.** a. The distribution of SCCs between experimental and predicted binding
11 affinity from trained (black) and tested (red) models of AA2AR, DHI1, and AL5AP.
12 The green arrow indicated the SCC of the complete dataset. b. Distributions of
13 experimental (black) and predicted (red) binding affinity of drugs to AA2AR, DHI1,
14 and AL5AP. c. Number of experimentally-validated drugs of AA2AR, DHI1, and
15 AL5AP in ascending rankings of predicted binding affinity. The red and blue bars
16 showed the experimentally-validated drugs in the corresponding ranking are
17 significantly over- and under-represented, respectively (Fisher's exact test, p -value <
18 0.05).

19 **Figure 2. The association between number of experimentally-validated drugs and**

20 **model performance.** a. Distribution of the number of experimentally-validated drugs
21 for proteins. Blue and red bars showed the proteins with experimentally-validated
22 drugs less and more than ten, respectively. b. Positive correlation between the drug

1 numbers and prediction SCC transformed z-score of models. c. Negative correlation
 2 between the drug number and standard deviation of SCC. The standard deviation was
 3 calculated from the SCCs between the experimentally-validated and predicted binding
 4 affinity from 1,000 training model. d. Association between the drug numbers and
 5 prediction stability. The prediction stability here is estimated by the difference of SCC
 6 between the complete dataset and training set. (E) Average SCC z-score of models
 7 predicting drugs of other proteins. Most of the models were unsuitable to predict the
 8 binding interaction targeting proteins other than themselves.

9 **Figure 3. The SCC of model coefficients and the mean drug number of model**
 10 **pairs.** For each protein pair, the SCC of model coefficients and average drug number
 11 were calculated. The green line indicated where the smallest significantly positive
 12 SCC (value ≥ 0.15 , p -value < 0.05).

13 **Figure 4. The functional similarity of the shortest paths in the protein similarity**
 14 **network.** a. The original network based on protein proportionalities. b. The auxiliary
 15 eBC network of the 556 proteins. For the primeval network, the proteins were
 16 fully-connected and could not be interpreted with corresponding biological
 17 similarities. After removing connections with low edge betweenness centrality, the
 18 network had positive correlation with GO term similarities and showed noticeable
 19 structure. c. The protein pairs with a two-step distance had the highest GO similarity

1 among pairs with each shortest paths. This implied that the indirect connections

2 between proteins could better reflect the biological similarities.

3

4

A

B

C

A **B** **C** **D** **E**

Means of Drug Number and Coefficients SCC of Model Pairs

A

B

C