Machine Learning Scoring Functions for Drug Discoveries from Experimental and Synthetic Protein-Ligand Structures: Towards Per-target Scoring Functions

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Abstract

In recent years, machine learning has been proposed as a promising strategy to build accurate scoring functions for computational docking finalized to numerical empowered drug discoveries. However, the latest studies have suggested that over-optimistic results had been reported due to the correlations present in the experimental databases used for training and testing. Here we investigate the performance of an artificial neural network in binding affinity predictions, comparing results obtained using both experimental protein-ligand structures as well as larger sets of synthetic structures created using commercial software. Interestingly, similar performances are obtained on both databases. We find a noticeable performance suppression when moving from random horizontal tests to vertical tests performed on target proteins not included in the training data. The possibility to train the network on relatively easily created synthetic databases leads us to explore per-target scoring functions, trained and tested ad-hoc on complexes including only one target protein. Encouraging results are obtained, depending on the type of protein being addressed.
Scoring functions (SF) are effective models used in computational docking to score poses of candidate ligands in the pockets of a target protein [11, 12]. They are expected to attribute the best score to the correct pose, to accurately predict the binding affinity (or a score proportional to the affinity), and to prioritize active ligands over inactive ones. The latter task, in particular, plays a pivotal role in virtual screening [3]. It allows identifying the most promising drug molecules, thus reducing the time and the cost otherwise spent for in-vitro screening [4]. Due to their importance in drug discovery [5], the development of SFs has been the focus of intense research endeavors for decades. Modern SFs are often grouped into empirical [7], knowledge-based [8], and force fields [9] SFs. More recently, SFs have been implemented also using data-based approaches via machine-learning (ML) techniques [10–12]. This strategy is favored by the increasing amount of crystallographic protein-ligand structures [13], and it aims at exploiting the effectiveness of ML techniques in extracting useful information from big databases. An influential article was published in 2010 [14]. Therein, the authors adopted a relatively simple ML model, namely, random forest regression [15], and they trained it to predict binding affinities using about 1200 protein-ligand complexes extracted from the PDBBind databases [16, 17]. They reported a promising Pearson correlation coefficient $R_p = 0.776$, which indicates a strong correlation between predicted and experimental binding affinities [18]. Notably, the ML-based SF outperformed all considered classic ones. Later, a similar ML-based SF was specialized to virtual screening tasks, thus also addressing the comments of Ref. [19] on the poor performance of ML-based SFs in this task. Various subsequent studies have adopted more advanced ML models, such as dense neural networks (NNs) [20], convolutional NNs [21–24], and graph NNs [25], often further increasing the performance in binding affinity prediction. For a recent review on neural networks and deep learning techniques, see, e.g., Ref. [26]. Different complex representations have been explored, ranging from distance-counts of protein-ligand atomic pairs (combined with kernel ridge regression) [13, 27] to three-dimensional (3D) grids of atomic features (combined with convolutional NNs) [21, 24]. However, very recent studies have put these optimistic results into question [28]. They reported a drastic performance reduction when ML SFs are subjected to more stringent benchmarks. Noticeable examples are so-called vertical tests, whereby predictions are made for proteins not included in the training set, as opposed to the more common and less stringent horizontal tests, whereby a protein might be present both in the training and in the test sets, albeit bound to a different ligand. Along this line, Refs. [22, 27] considered scaffolding tests designed to quantify how the prediction accuracy varies
with the degree of structural similarity between the proteins in the training and in the test sets. Notably, Ref. [28] reported that the performance of an exemplary ML-based SF did not change when it was fed with only the protein structure, or only the ligand structure (as opposed to the whole complex). The authors thus suggested that the ML-based SF had not learned any information about the actual protein-ligand binding mechanism.

A critical problem for the development of ML techniques for computational docking is the limited number of experimental structures available for model training [18, 29]. From the most relevant database, namely, the PDBBind repository, one may extract a few thousand complex structures, depending on the required resolution [29]. While this number is steadily increasing, it is still orders of magnitude smaller than the amount of data typically used in other fields where ML has proven astonishingly successful, such as, e.g., computer vision. For example, state-of-the-art NNs for object detection are usually trained on databases including millions of images, e.g., the ImageNet database [30]. This issue is particularly relevant for deep NNs. These are often preferred to simpler ML regression models, such as kernel ridge regression or support vector machines, due to their superior generalization properties. However, in general they require larger training databases to avoid overfitting problems. The contrasting findings discussed above call for further quantitative performance analyses on ML-based SFs, considering in particular the role of the training set and of the test type. One of the main questions we aim to address in this Article is whether ML-based SFs can be trained using synthetic complex structures created using docking software, providing access to larger and more tunable databases.

In this Article, we implement a SF using a NN with fully connected layers (FCNN). Following Refs. [14, 27], the descriptors used to represent the complex structures are counts of atomic pairs, one belonging to the protein and the other to the ligand, within various distance intervals. Two databases are used for training and testing. The first includes 2408 crystallographic structures extracted from the PDBBind database. Notably, we carefully check and prepare these structures with the inclusion of hydrogen atoms, in contrast to previous related studies who only considered heavy atoms. The second database includes 28,200 synthetic structures created using the CCDC Gold docking engine within the MOE (Molecular Operating Environment) software interface [31–33]. In both databases, the complex structures are associated to the corresponding experimental binding affinities. The FCNN is trained to predict binding affinities of previously unseen complexes via a supervised learning algorithm. One of our goals is to quantify the performance of a FCNN combined with a distance-count description of protein-ligand complexes. Chiefly, we compare the performances reached using experimental and synthetic databases, considering in both cases both
horizontal as well as more challenging vertical tests. Finally, taking advantage of the creation of synthetic databases, we explore the development of per-target SFs designed to predict binding affinities for a specific target protein. We consider 17 exemplary targets, training a specific SF for each of them using synthetic databases including many complexes with different ligands docked into the same target protein. The performances obtained on the exemplary targets are analyzed, also varying the size of the training set. In general, the obtained performances are encouraging, with noticeable variations depending on the target. To shed some light on these different performances, we make comparison against basic linear regression models based on the molecular weight, optimized for each target.

II. METHODOLOGY

A. Experimental database

The first database includes 2408 complex structures measured through X-ray crystallography [34] and deposited into the Protein Data Bank [35, 36]. We select structures with a resolution degree lower than 3Å and whose ligand-target interaction information is available from the PDBbind database [16, 17]. The 3D structures are manually checked for possible inconsistencies and curated with the addition of the hydrogen atoms, using the MOE software. This process is time consuming, thus limiting the size of the experimental database. However, it allows us adopting a more complete representation, as opposed to various previous related studies who considered only heavy atoms. Furthermore, the inclusion of hydrogen atoms allows performing a more direct comparison against the synthetic complexes described below. The target-ligand structures are associated with the corresponding dissociation constant $K_d$. In fact, we consider the value of $pK_d$, defined as:

$$pK_d = -\log_{10} (K_d) = -\log_{10} \left( \frac{[P][L]}{[C]} \right),$$

where $[P]$, $[L]$, and $[C]$ represent the concentrations of the protein, of the ligand, and of the complex, respectively. Some relevant details on our databases are summarized in Table I.

| Database      | Number of complexes | Mean affinity | Mean docking score |
|---------------|---------------------|--------------|--------------------|
| Experimental  | 2408                | 5.98 ($pK_d$) |                    |
| Synthetic     | 28200               | 7.48 ($pK_i$) | 11.43              |

TABLE I. Brief description of the experimental and the synthetic databases.
B. Synthetic database

The second database is synthetic. To build it, the 3D structures of 17 selected target proteins are retrieved from the PDB repository. The selection focuses on diverse targets with many ligands deposited in the BindingDB database \[37–39\]. Only complexes with a single ligand at the binding site and no cofactors are considered. For each target, a numerous set of ligands is retrieved. The choice of ligands is restricted among those whose experimental binding affinity for each selected target is available as expressed by the \( pK_i \) score. This score measures the target-ligand affinity using a reference radio-ligand. Its value is generally close to \( pK_d \). These ligands are docked into the respective target using the GOLD docking engine through the MOE software. Our simulations produce ten poses for each target-ligand pair, and the one corresponding to the best docking score is selected. In total, the synthetic database includes 28200 protein-ligand complexes. The number of pairs per target ranges from 384 for the PIM2 protein, to 6568 for the D2 protein. The selected target proteins and the corresponding number of complexes are summarized in Table II. Evidently, the synthetic database is significantly larger than the experimental one. This allows us to better analyse the learning speed of NNs. However, it is worth emphasizing that the docking pose created by the docking software is affected by the possible inaccuracy of the chosen docking engine. Instead, the 3D crystallographic structures are expected to correspond to the actual spatial configuration. Spurious distortions might be introduced by the measurement procedure, but this is believed to rarely happen. It should be noted, however, that this does not necessarily represent a drawback. Indeed, in virtual screening campaigns, SFs are often used to select promising ligands from software-generated poses. Therefore, training the SF on the type of structures it will be asked to rank might actually be instrumental.

| Protein | 5HT2A | A2A | BACE1 | DOP | FAAH | GR | H1 | JAK1 | PI3K |
|---------|-------|-----|-------|-----|------|----|----|------|------|
| N. of complexes | 2763 | 2914 | 1413 | 1243 | 508  | 843| 1070| 1213 | 1064 |

| Protein | PIM2 | ACE | KOP | M1 | MCL1 | JAK2 | OX2 | D2 |
|---------|------|-----|-----|----|------|------|-----|----|
| N. of complexes | 384 | 488 | 2431 | 1056 | 688  | 1394 | 2160| 6568 |

TABLE II. Breakdown of the protein-ligand complexes included in the synthetic database.

C. Complex representation

The input provided to the FCNN must be designed to represent, with good approximation, the 3D structure of the protein-ligand complexes. The description we adopt follows the archetypes
proposed in previous studies \[14, 27\]. Specifically, each descriptor corresponds to the count of atom pairs within a specified distance interval, whereby the first atom belongs to the target, the second to the ligand. The following 10 atomic species are considered for the target atoms: H, C, N, O, F, P, S, Cl, Br, and I. For the ligands, only the species H, C, N, O, P, and S are considered. This restriction is due to our choice of addressing only ligands not including metallic atoms. This corresponds to 60 descriptors per distance interval. Notably, our representation takes into account H atoms. This is in contrast to the previous related studies, which only considered heavy atoms. Furthermore, originally Ref. \[14\] considered only one distance interval, namely, 0-12Å. Subsequently, Ref. \[27\] adopted a more detailed representation, dividing the 12Å range into six intervals. In this article, we explore different representations, varying both the number of intervals and their width. The goal is to identify the optimal compromise between representation accuracy and conciseness. We point out that the original training matrix includes descriptors which might differ by order of magnitudes. For this reason, a normalization operation is helpful. Our analysis on the experimental database shows that the most effective operation is dividing by the maximum value of all descriptors. This normalization is adopted for all results reported below.

D. Target values

SFs are designed to predict a score proportional to the binding affinity. As already mentioned, we train and test our FCNN SF using, as regression target, the $pK_d$ and the $pK_i$ values, for the experimental and the synthetic databases, respectively. For the latter database, in some analyses we also consider the docking score. For convenience, we adopt as target value the negative of the docking score, so that higher values correspond to putatively higher affinities. The mean target values of our databases are summarized in Table I.

Notice that, during training, we adopt the standardized targets $d' = \frac{d - \mu}{\sigma}$, where $d$ is the original value, $\mu$ the database mean, and $\sigma$ the corresponding standard deviation. This (linear) standardization does not affect the correlation coefficient $R_p$. Furthermore, to favor comparison with other studies, the mean squared error (MSE) values reported below are obtained considering un-normalized predictions and targets $d$, obtained by inverting the standardization formula.
E. Regression model and training protocol

The goal of the supervised learning is to train a regression model to map the complex descriptors to the target value, namely, the binding affinity or the docking score. The regression model adopted in this article is a FCNN, namely, dense NN with all-to-all interlayer connectivity. The numbers of (hidden) layers \( N_l \) and of hidden neurons per layer \( N_h \) are chosen through the analysis described in Section [III]. Note that \( N_l \) does not count the descriptor layer, nor the output layer featuring a single neuron. The activation function in the hidden layers is the hyperbolic tangent. The network weights and biases are optimized by minimizing the loss function, namely, the MSE between network’s predictions and the ground-truth target values. To contrast possible overfitting phenomena, the loss function is augmented with a standard \( L_2 \) regularization term. However, this does not lead to significant benefits, and the results we report hereafter correspond to a negligibly small regularization parameter. The optimization is performed using a variant of stochastic gradient descent, named ADAM [40]. An adequate mini-batch size turns out to be around 50 and 200, for the experimental and the synthetic databases, respectively.

The training epochs are iterated until the prediction accuracy on the test set stops improving, i.e. before entering the regime where overfitting phenomena dominate. Due to the small size of the experimental dataset, introducing a validation set is not practical. For consistency, the same protocol is adopted also for the synthetic dataset. This is intended to estimate the optimal potential performance. While, in principle, it might lead to a slight overestimation, this effect is found not to be important. In particular, in the most critical per-target FCNN SF tests, the prediction accuracy is found to be quite stable as a function of the training epochs. To monitor the prediction accuracy, two metrics are considered, namely, the Pearson correlation coefficient \( R_p \) and the MSE. These are computed on a test set which includes around 20% of the whole database, while the remaining instances are used for training. The training and testing process is repeated ten times, considering just as many different random splittings between training and test instances, or simply different (random) selections of training data and mini-batches for gradient descent. The accuracy scores reported hereafter correspond to the average of the test scores, while error bars correspond to the estimated standard deviation of the average. This averaging procedure avoids spurious fluctuations due to accidentally favourable or adverse selections of the test instances, providing a more reliable estimate of the prediction accuracy in a realistic scenario.
FIG. 1. Pearson correlation coefficient $R_p$ for the predictions of binding affinity provided by the FCNN SF as a function of the number of descriptors $N_f$. This FCNN SF is trained and tested on the experimental dataset. The protein-ligand complex structure is represented by atomic-pair counts within a variable number of distance intervals. The three datasets correspond to different interval widths. 60 pairs of atomic species are considered, including pairs with hydrogen.

III. RESULTS

A. Selection of descriptors and network structure

To identify the optimal choice for the complex-structure representation, we analyse the prediction accuracy on test sets of 300 randomly chosen experimental complexes, as a function of the number of distance intervals of atom-pair counts. Notice that, while the test protein-ligand complexes are distinct from those used for the optimization of weights and biases, some target proteins might occur in both training and test sets, albeit bound to a different ligand. This is what we refer to as a horizontal test. The results are shown in Fig. 1 considering three interval widths, namely, 1.5Å, 2Å, and 3Å. The optimal representation includes four intervals of width 2Å, meaning that atom pairs are counted only if the two atoms are less than 8Å apart. Considering that 60 pairs of atom species are considered, the total number of descriptors is 240. This representation is adopted for all results reported hereafter.

We identify the optimal depth and width of the neural network by analysing the $R_p$ score on the experimental test set. The results are shown in Fig. 2 as a function of the number of training
FIG. 2. $R_p$ score for binding affinity prediction as a function of the size $N_t$ of the (experimental) training set. Each dataset corresponds to a choice $N_l \times N_h$ for the number of hidden layers $N_l$ and of neurons per layer $N_h$ in the fully connected neural network (FCNN). The complex representation includes $N_d = 60$ descriptors, with 4 distance intervals of width 2Å.

instances $N_t$. Different numbers of layers $N_l$ and of neurons per (hidden) layer $N_h$ are considered. The highest performance is obtained for $N_l \times N_h = 2 \times 20$. An analogous analysis performed on the synthetic data (not shown) indicates that, in that case, the optimal network structure is $N_l \times N_h = 4 \times 40$. The need of a deeper network can be attributed to the larger size of the synthetic database. Indeed, it is known that deeper NNs are more effective in extracting useful information from larger databases, while they are more susceptible to overfitting phenomena when the database is sparse. These two NN structure are adopted for all results reported below, unless otherwise specified.

B. Horizontal tests on experimental and synthetic structures

One of our main goals is comparing the performances of ML-based SFs trained and tested on experimental and on synthetic databases. The two learning curves for our FCNN SF are compared in Fig. 3, where the $R_p$ score for the binding affinity prediction is plotted as a function of the training set size $N_t$. As a term of comparison, the $R_p$ value obtained when the (negative) docking score is used as target value, both during training and testing phases, is also shown. Noticeably, remarkably high performances are obtained in this latter test, namely, $R_p \approx 0.82$. This indicates that the chosen combination of representation and regression models is capable of learning, within
FIG. 3. $R_p$ prediction-accuracy score as a function of the training set size $N_t$. The red rhombi and the green squares correspond to the binding affinity prediction by the FCNN SF trained on the experimental complexes and on the synthetic databases, respectively. The blue triangles correspond to the predictions of the docking score by the FCNN SF trained on the synthetic database. The network structure is described in Figures 1 and 2.

FIG. 4. $R_p$ prediction-accuracy score as a function of the percentage of the training set size $N_t$ compared to the whole database size $N$, namely, $100N_t/N$. As in Fig. 3, the red rhombi and the green squares correspond to the FCNN SF trained on the experimental complexes and on the synthetic complexes, respectively.
FIG. 5. Mean squared error (MSE, upper panel) and \( R_p \) score (lower panel) for binding affinity prediction in the vertical test, as a function of the training set size \( N_t \). The FCNN SF is trained on a synthetic database including complexes made from 13 proteins, and tested on complexes made from 4 proteins not included in the training set.

A good approximation, the function corresponding to the docking engine. The maximum \( R_p \) score corresponding to the binding affinity prediction is somewhat smaller, namely, \( R_p \simeq 0.55 \) and \( R_p \simeq 0.60 \), for the experimental and the synthetic databases, respectively. These scores still correspond to moderately strong correlations between predictions and ground-truth (i.e., experimental) binding affinities. The lower score at the maximum \( N_t \) on the experimental database can be attributed to the larger size of the synthetic one. Actually, it appears that the learning is faster on the experimental database, since higher accuracies are in fact obtained when the training set size \( N_t \) is comparable.

To further inspect this effect, the same \( R_p \) scores are plotted in Fig. 4 as a function of the percentage of training instances compared to the size \( N \) of the whole database. One observes an approximately linear increase. The slopes corresponding to the experimental and to the synthetic databases are comparable. This suggests that the performance improves due to the increasing probability of finding the same or similar proteins in both the training and the test sets. Consistently with this supposition, the \( R_p \) score is consistently higher on the synthetic database, whereby the number of proteins is lower. A similar supposition has been put forward in Ref. [28], and it is supported also by the vertical tests discussed below.
C. Vertical tests

The horizontal tests described above provide encouraging results. As anticipated, however, these might be over-optimistic, being biased by the similarities among the complexes present in the training and in the test sets. In a real-case scenario, universal SFs are expected to describe the binding strength of candidate ligands into novel proteins under investigation. Quite likely, these proteins are dissimilar from those included in the databases available at the time of model definition. A fairer performance assessment is therefore provided by so-called vertical tests, whereby complexes made from proteins present in the test set are excluded from the training set. The first vertical test we consider is performed on synthetic complexes made from the four proteins FAAH, PIM2, ACE, and MCL1, totaling 2068 complexes. The FCNN SF is trained on \( N_t \) complexes made from the remaining 13 proteins of our synthetic database (see Table II). The \( R_p \) score is shown in Fig. 5 as a function of \( N_t \). One notices a significantly reduced performance compared to the horizontal test. The accuracy score, \( R_p \approx 0.4 \), corresponds to an only moderate correlation between predicted and experimental binding affinities. It is worth mentioning that a similar vertical test on synthetic complex structures was performed also in Ref. [27] using a random-forest regression model. That study reports an even lower score, namely, \( R_p \approx 0.2 \). We attribute the better performance of our FCNN SF to the use of an FCNN, which is more suited for extracting useful information from
FIG. 7. $R_p$ score for binding affinity predictions from six per-target FCNN SF’s, as a function of the training set size $N_t$. Each SF is trained and tested on synthetic complexes made from the protein indicated in the legend.

large databases. More importantly, however, the performance we observe does not improve with the training set size $N_t$. To further elucidate this finding, we perform a series of per-target vertical tests. 17 FCNN SF’s are trained on just as many synthetic datasets obtained by excluding all complexes made from each of the 17 proteins. The excluded complexes are used as test sets for the corresponding SF. The 17 corresponding $R_p$ scores are shown in Fig. 5. Again, the performance is, on average, appreciably lower compared to the horizontal test discussed above. This corroborates the claim that horizontal tests provide over-optimistic performance measures, probably due to the correlations and similarities among training and test complexes. Also noteworthy are the large performance fluctuations among the 17 FCNN SF’s corresponding to the different targets. For example, remarkably high scores are obtained for the JAX1 and JAX2 proteins. These might be attributed to the similarity between these two proteins, meaning that including in the training set complexes derived from one of them allows the FCNN SF learning how to predict binding affinities for the other. However, also the close relationship between binding affinity and ligand molecular weight might play a role. This is further discussed below.
D. Per-target scoring functions

The unremarkable performances displayed by the universal ML-based SF in the vertical tests lead us to explore different strategies. As discussed in Section III B, the experimental and the synthetic databases appear to be comparably effective for training SFs for binding affinity predictions. Clearly, the synthetic complexes can be relatively easily generated. This suggests the idea of developing per-target SFs on-demand, whenever a novel protein is targeted. Such a SF would be trained on a purposely created database including only complexes made from the target protein. To explore this direction, we consider the six proteins with more complexes in our synthetic database, namely, D2, A2A, 5HT2A, KOP, OX2, and JAX2. Six per-target FCNN SF’s are trained and tested only on complexes made from one protein. Motivated by the sizes of the six databases, we adopt FCNNs with $N_l \times N_h = 2 \times 20$, apart for the D2 target, for which the parameters $N_l \times N_h = 3 \times 20$ are expected to be more adequate. The corresponding $R_p$ scores for binding affinity prediction are shown in Fig. 7, as a function of the training set size $N_t$. The tests are performed on 300 complexes. The performances are relatively high. The average $R_p$ obtained using, for each target, $N_t = 900$ training instances, is $R_p = 0.44$. When the largest available $N_t$ for each target is employed, the average score is $R_p = 0.52$. These scores are to be compared with the average vertical test on these six targets, corresponding to $R_p = 0.30$. Chiefly, in all six tests the $R_p$ score systematically increases with $N_t$, suggesting that sufficiently performant per-target SFs can be obtained when adequately large synthetic training databases are available. There are also noticeable performance differences among the six targets, ranging from $R_p \approx 0.4$ for D2, to $R_p \approx 0.67$ for JAK2. To shed some light on these differences, we compare the per-target FCNN SF’s to simple linear regression models. Specifically, we assume the linear law $pK_i = A + mB$, where $m$ is the ligand molecular weight (MW), and $A$ and $B$ are the fitting coefficients. These are fixed via MSE minimization on the whole per-target database. The comparison between the per-target FCNN SF and the corresponding MW SF is shown in Fig. 8 for two exemplary targets, namely the JAK2 and OX2 proteins. Notice that the corresponding scores in the per-target vertical tests are also shown. One notices that for the JAK2 protein even the simple MW SF provides a remarkable performance, namely, $R_p \approx 0.62$. This might be tentatively attributed to the large pocket size at the binding site for this particular protein, meaning that the binding strength is simply proportional to the ligand size. It is, therefore, not surprising that both the per-target FCNN SF and the universal FCNN SF in the per-target vertical test provide comparable (but still superior) scores. Instead, for the OX2 protein the predictions of the MW SF have essentially zero correlation with the binding affinity,
FIG. 8. $R_p$ accuracy scores as a function of the training set size $N_t$, for two exemplary target proteins, namely, JAK2 (cyan) and OX2 (red). The performances of the per-target FCNN SF’s (cyan triangles for JAK2 and red circles for OX2) are compared to the corresponding scores of the FCNN SF in the vertical test (cyan full diamond and red full square) and of the molecular weight linear regression (cyan empty diamond and red empty square). The large green x’s correspond to the universal FCNN SF in the horizontal test on the experimental database, while the small blue x’s correspond to the analogous test of the synthetic database. The horizontal gray line represents the average score on the 17 per-target vertical tests.

corresponding to $R_p \approx 0$. The per-target FCNN SF reaches an encouraging $R_p \approx 0.53$ at the largest $N_t$. Notably, this per-target FCNN SF significantly overcomes the score of the universal FCNN SF in the corresponding per-target vertical test ($R_p \approx 0.18$). These findings suggests that per-target FCNN SF’s are able to learn non-trivial mappings from computationally feasible synthetic databases.

IV. DISCUSSION

We have analysed the performance of SFs based on FCNNs in predicting the binding affinities of protein-ligand complexes from a representation of the 3D complex structure. Our choice for the structure representation is based on atomic-pair counts within suitably chosen distance intervals, and we investigated the optimal number and width of such intervals. The effectiveness of crystal-
lographic 3D structures for SF training has been compared to that of synthetic structures created with widely available docking simulations software. Notably, the two types of data turn out to be comparably effective, suggesting that more accessible synthetic database can be used for SF development, rather than the less copious experimental structures. Importantly, our FCNN SF’s have been assessed both in horizontal tests and in vertical tests, whereby no protein is included both in the training and in the test databases. A significant performance degradation is found in the latter, as in fact reported also in Ref. [27] using different regression models compared to the NN adopted here. This corroborates the contention of Ref. [28], where it is argued that ML-based SFs might not learn the ligand-target binding mechanism, but rather the correlations among complexes present in the training and in the test sets. These findings indicate that vertical tests represent fairer assessments for the performances of ML-based SFs in real-case scenarios. The relative easy of creating synthetic databases led us to explore the development of per-target FCNN SF’s. Six exemplary targets have been considered, obtaining encouraging results. Chiefly, a systematic performance improvement with the training set size was observed. Furthermore, we found instructive results by comparing the FCNN SF’s to linear-regression models based on the molecular weights. Interestingly, in some cases even such simple theories reach high correlation with experimental binding affinities.

SFs are an important tool to accelerate drug discovery. Massive research endeavours have been devoted to implement different families of SFs. However, the development of SFs based on ML is still in an early stage. The variable and sometimes contrasting results reported in the literature indicate that more research is due to establish reliable benchmarking protocols. To facilitate future comparative studies, we provide our databases (experimental and synthetic) via Ref. [41], including the 3D structures, deposited in PDB files, as well as the binding affinities and the docking score.

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