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ORIGINAL PAPER

Using protein-ligand docking to assess the chemical tractability of inhibiting a protein target

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Abstract Assessing the difficulty of inhibiting a specific protein by a small molecule can be highly valuable in risk-assessment and prioritization of a new target. In particular, when the disease linkage for a number of targets is broadly similar, being able to identify the most tractable can have a significant impact on informing target selection. With an increasing focus against new and novel protein classes, being able to assess the most likely targets to yield lead-like chemical start points can guide the selection and the lead-generation strategy implemented. This study exploits protein-ligand docking studies on published protein x-ray crystal structures to provide guidance on the feasibility of identifying small molecule inhibitors against a range of targets.

Keywords Druggability · Ligandability · Protein-ligand docking

Introduction

It has been reported that 60% of small-molecule drug discovery projects fail due to the lack of suitable leads [1]. The term ‘druggability’ has subsequently been used to capture the feasibility of inhibiting a specific target with an orally bioavailable small molecule [2]. Some estimates indicate that only 10% of genes in the human genome are ‘druggable’ and only 5% are druggable and relevant to disease [3]. The druggability of a target is currently assessed in a number of ways, this includes studying known inhibitors from the gene family and fragment/subset screening [4]. Fragment and subset screening have been deployed against targets to identify the ease of finding small molecule binders which can then indicate the likelihood of success by HTS and or random screening. The data from these studies is encouraging although some targets may not be suitable for such screening campaigns and also significant resources are required to carry out this work on a set of proposed targets in a timely fashion. Analyses of a target binding site can also be exploited where suitable crystal structures are available [5, 6]. Such analyses can utilize information on the size, concavity and electrostatic nature of a pocket; one such recent report translates this information into a maximal affinity model [7]. This study highlights how a target with a well-defined pocket with a suitable balance of properties will score more highly than an open and feature-less pocket which can be observed in some protein-protein interactions. This approach is restricted to targets which have suitable protein crystal structures but encouragingly new and novel x-ray crystal structures continue to be published [8]. Protein movement and flexibility is also an issue as some pockets are only fully formed when a ligand is bound [9]. Finally, analysis of the protein family or class can be used to estimate druggability. So for example if a proposed novel target is a kinase and a related family member has been shown to be tractable you might assume the druggability is likely to be comparable [10]. Clearly if a novel protein family or target class is being assessed there may be little information in the literature to provide this guidance.

The study detailed in this paper uses a test-set of 950 diverse fragment-sized molecules [11] to allow an *in silico* druggability prediction through docking against a target binding site. This can be considered as the computational equivalent of a fragment screen, but where a specific pocket

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is targeted by each run. These fragments have been filtered using previously reported criteria and largely consistent with reported fragment library characteristics [11, 12]. Although docking scores do not consistently show a correlation with ligand binding affinities [13], the expectation is that a druggable binding site is likely to score fragments more highly when productive hydrogen bonding and lipophillic interactions with the protein can be identified. This measure of assessing the druggability of a binding site might be more accurately described as 'ligandability', where a protein of high ligandability is likely to bind fragments with a reasonable affinity. Identifying ligands which bind to a protein may not necessarily translate into finding a drug if the nature of the binding site does not allow the affinity of the lead to be increased whilst maintaining the required drug-like properties [14]. However, an indication that fragments can be identified for a target protein gives some confidence that the target might be tractable and or a tool compound may be identified for target validation work. Additionally, if this information could be obtained computationally it would allow a greater number of targets to be considered than using experimental approaches.

Materials and methods

Fragment set

A set of 950 diverse fragments were identified for this study. The distributions of ClogP (Version 4.3), molecular weight and rotatable bonds are shown for this fragment set below in Fig. 1.

The fragment set has a mean ClogP of 1.6 and the mean molecular weight was 188. The number of rotatable bonds in the set is centered on two, with a range between 0 and 6. The number of hydrogen bond acceptors and donors were also kept relatively low. Compounds with functionality not compatible with good fragment leads were also removed from the set. The reasoning behind using a fragment set filtered to these criteria was to provide consistency to the druggability assessments which have been run using experimental approaches on similarly filtered fragment libraries [4].

Protein preparation

The publicly available protein structures used in this study were taken from the RSCB Protein Data Bank [15]. The proteins were prepared for docking and analysis using the Protein Preparation Wizard in Maestro [16]. Hydrogens were added, reflecting the likely protonation states of amino acids at pH=7.4. The hydrogen bonding environments of amino acids such as Asn and His were analyzed to ensure that the most likely conformation had been assigned [17]. Unless an interface between dimer/tetramer units was required for binding a single monomer unit was used for the docking. Where a suitable ligand/co-factor was present in the pocket, it was used to define the binding site for the docking. Where no ligand was present, SiteMap [18] was used to identify probable binding sites, the primary pocket was then manually defined using amino acid residues.

Protein-ligand docking

The likely protonation and tautomeric states of the 950 compound set was enumerated in Leatherface [19]. This produced around 1150 distinct chemical structures. A 3D-structure of each molecule was produced in CORINA [20], which was then optimized using the MMFF94 Forcefield [21], and the Sheffield solvent model [22]. These structures were then used as input into the docking calculations. Glide was used for the protein-ligand docking [23]. Standard precision (SP) and extra precision (XP) docking protocols were run for comparison. The highest scoring pose for each compound (which includes the various protonation/tauto-

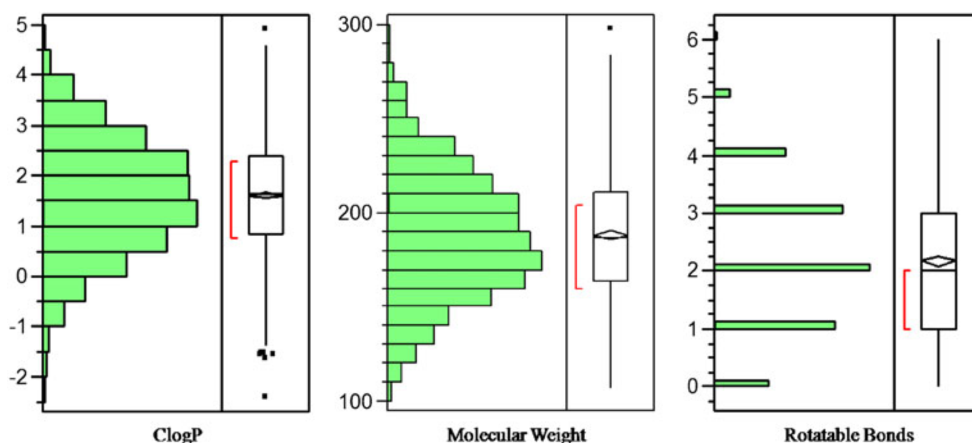

Fig. 1 Property profile of fragment set for 'ligandability' studies using docking

meric states) was used to represent the score for that compound against the target.

Protein structures

The following tables show the x-ray crystal structures used for the docking studies. PDB codes of proteins from the RCSB protein data bank are provided [15].

Results and discussion

Correlations of docking score against physiochemical properties

It has been demonstrated that docking scores can correlate with the molecular size of the compounds docked [13]. To ensure that this was not the case in this study the mean docking score of each fragment was calculated across all the protein targets studied. This value was then plotted against molecular weight and ClogP from the Standard (SP) and Extra Precision (XP) docking protocols. Figure 2 shows that there is no significant correlation with the molecular weight or ClogP of the fragment and the mean docking score across the targets.

Interestingly, the XP protocol shows a stronger trend, albeit still a weak one, between the mean docking score of a fragment across the targets and molecular weight/ClogP (Fig. 2d). The RSquare=0.22 for XP plotted against molecular weight in Fig. 2d can be compared with an RSquare=0.08 with SP in Fig. 2b. The correlation of the docking scores against MW and ClogP was also examined for individual targets but there were no clear trends. Any assessed targets, which do show a significant correlation, may indicate that higher affinity fragments could be identified outside the property ranges in the fragment set.

Comparison work with maximal affinity prediction model (MAP_{POD})

This section of the study compared a number of the systems published in the maximal affinity model (MAP_{POD}) method to provide an assessment of how the two approaches compare with each other. The majority of studied targets classified as 'undruggable' and 'prodrug/transporter' were analyzed as these contained relatively small numbers of enzymes. The 'druggable' targets were then sampled from to enable a reasonable assessment against this approach. For a number of these targets additional protein structures have been considered (see Table 1) compared to the approach reported by Cheng et al. [7] to maximize the available binding site information. For this assessment, the mean docking score of all 950 fragments docked to the target protein was used as an

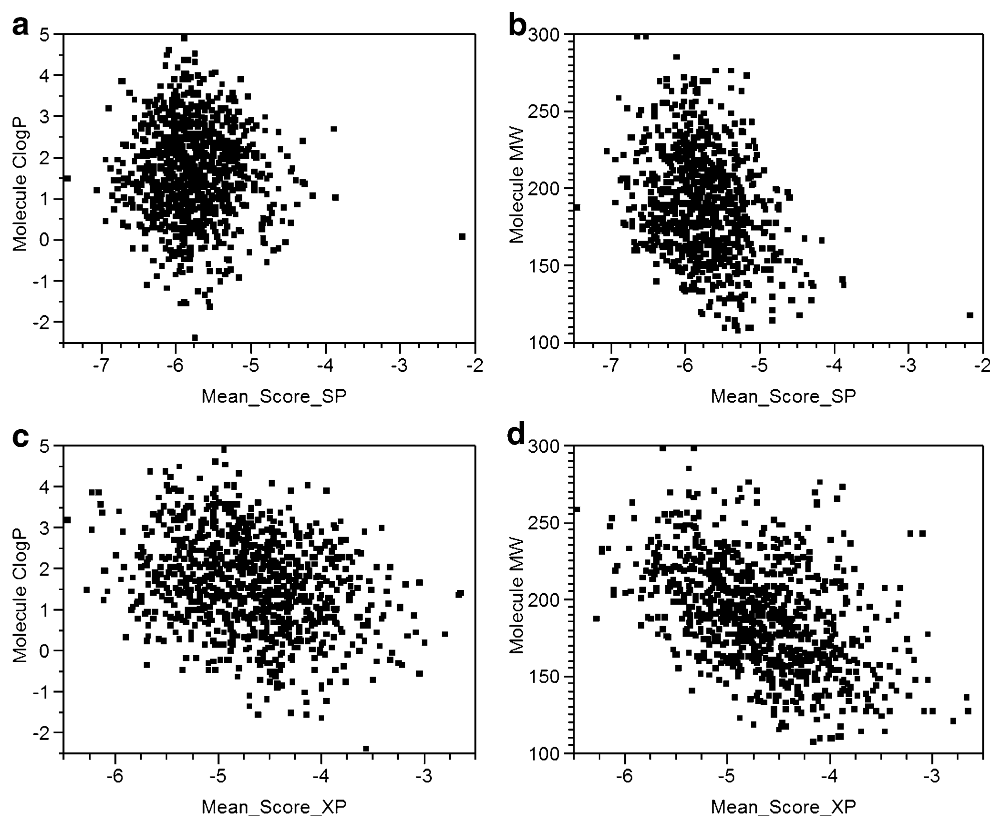

Fig. 2 Comparison of SP and XP protocol mean docking scores across targets against ClogP and Molecular Weight. 2a and 2b show the Glide SP results with 2c and 2d showing the XP results

Table 1 X-ray crystal structures used in ligandability assessment by docking

Protein name	PDB Codes
Enoyl reductase	1C14, 1DFI, 1ISZ
Aldose reductase	1PWL, 1T41, 1PWM
CDK2	1E1X, 1H07, 1H08, 1HCK, 1KE6, 1KE8, 1KE9
Cyclo-oxygenase 2	4COX
EGFR	1N1M, 1XKK
MDM2	1RV1
P38	1KV2, 2GFS
Acetylcholinesterase	1O86
IMPDH	1NF7
Thrombin	1KTS
CATK	1MEM, 1NLJ
ICE-1	1BMQ
PTP1B	1GFI, 1NNY, 1ONZ, 1PTY, 1Q1M, 1T4J

indicator of ligandability. Where multiple protein structures were used, the mean docking score across all the structures was taken. Note that a more negative docking score shows an improvement in the predicted affinity of the fragments. To assess how the ligandability prediction from the SP and XP docking protocols compare, they are plotted against each other in Fig. 3.

The analysis demonstrates that the SP and XP protocols correlate reasonably well (RSquare=0.89) and as XP is ~10x slower than SP it may not add additional significant value. However, it may be prudent to run both protocols as some targets may vary in their predictions and as Glide can be run using parallel processing the additional computational resource is small. Additionally, as these docking algorithms develop it is a useful crosscheck to ensure that they give a consistent view with previous observations. The SP and XP protocols were then compared to the published MAP_{POD} [8] for a subset of the previously published targets (Table 1), the MAP_{POD} was converted into a pKd for this comparison (Fig. 4).

The comparison demonstrates that there is some consistency in how you would classify these targets based on MAP_{POD} and the fragment docking protocol. However, the RSquare values are relatively low, 0.50 for SP and 0.55 for XP. Importantly, the lower scoring targets (PTP1B, ICE-1, IMPDH and CATK) are highlighted as difficult targets in both approaches. One most notable outlier between the two techniques is MDM2. This has been defined as a druggable target but it is clearly a challenging one due to the target binding site being a protein-protein interaction [24]. Aldose Reductase is an example in which the fragment docking protocol predicts the target to have a higher druggability than the maximal affinity model.

The consistency of the ligandability assessment across different protein structures of the same target were also analyzed (Fig. 5). Where possible multiple protein structures were used to assess the ligandability of a target. The data shows that the choice of structure does impact on the ligandability score although in most cases it would not significantly change your view of the target. The protein structures chosen for these assessments were chosen to maximize structural variation to give a more realistic view of the applicability of this method. However, it is clear that for some targets where a significant conformational change is required to form the binding site this approach will not successfully predict ligandability.

To highlight the impact of the crystal structure chosen, for P38 and EGFR, an example of an active and inactive crystal structure was used. This was a DFG-in and DFG-out structure for P38 (1GF2 and 1KV2 respectively) and a c-Helix in and c-Helix out structure (1N1M and 1XKK respectively) of EGFR. In both cases the inactive structures

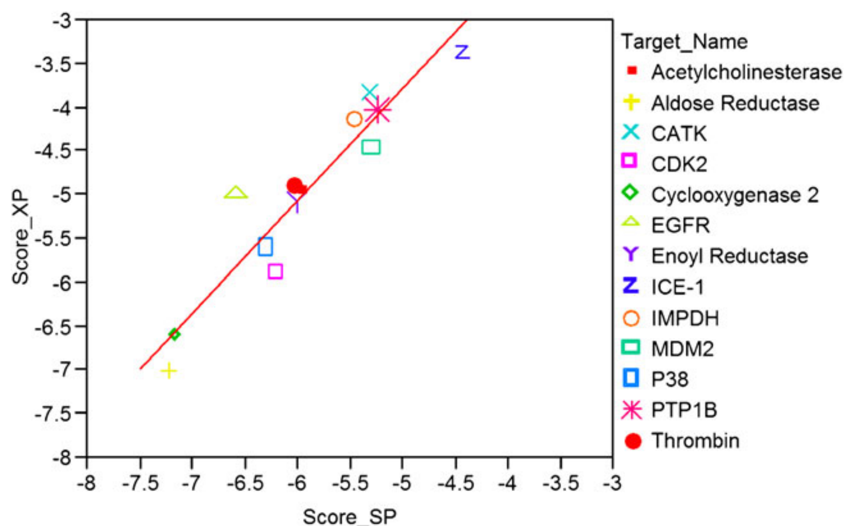

Fig. 3 Comparison of Ligandability prediction from SP and XP Glide docking. SP score (x-axis) against XP score (y-axis)

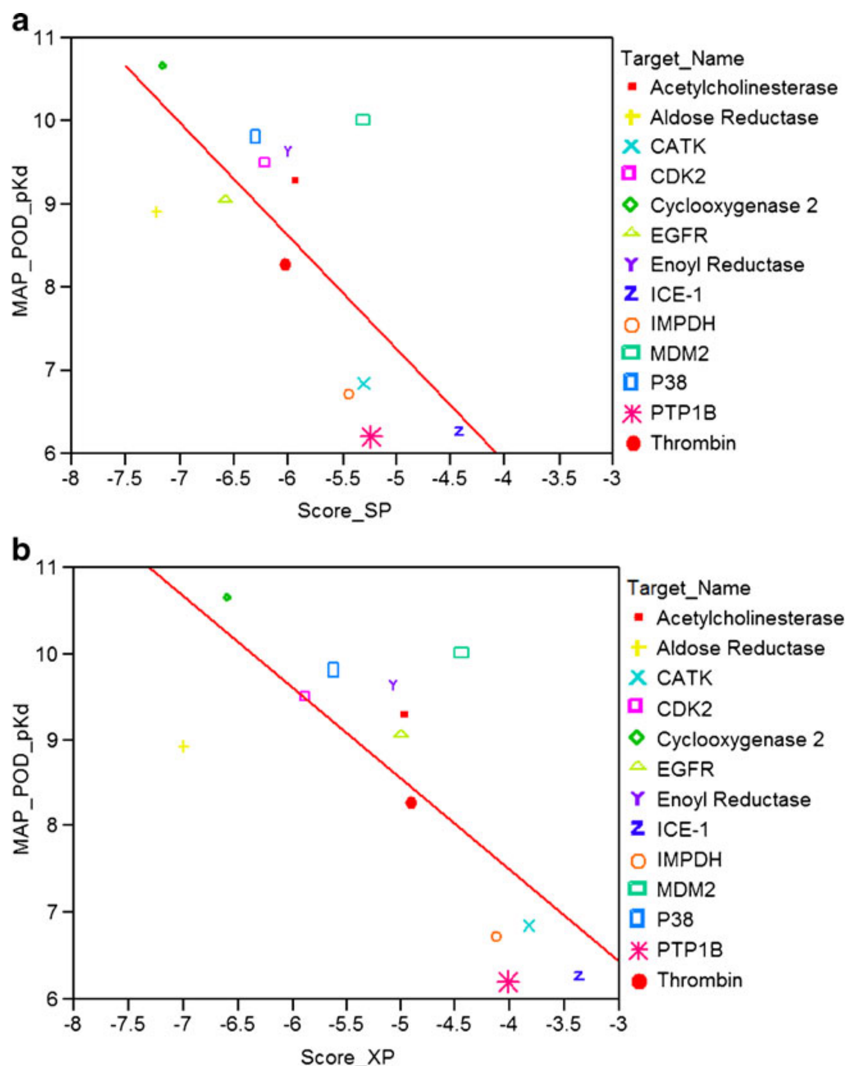

Fig. 4 Comparison of the fragment docking approach with the maximal affinity model (expressed in pKd). The SP protocol data is shown in 4a (RSquare=0.50), the XP data is in plot 4b (RSquare=0.55)

scored slightly higher, this appears to be at least partly due to the fragments having a choice of two sub-pockets to choose from. One of these regions is at the 'hinge' region and the other is in the 'selectivity pocket' of the kinase [26]. The inactive structures also allow fragments to be buried in the deep lipophillic selectivity pocket which is often scored favorably. All scores quoted below are from the SP protocol which was shown to track well with XP for a given target. In P38, the inactive structure had a docking score of -6.6 whereas the score was -6 for the active conformation. In EGFR, the inactive structure had a score of -6.6 compared to the active structure with a score of -5.9.

For the study of enoyl reductase [27], three crystal structures were used with four docking protocols. The structures 1C14 and 1ISZ both have cofactor and an inhibitor bound, the inhibitor binds to the protein-cofactor complex. In these systems the inhibitor was removed and docking carried out against the protein-cofactor complex. Fragment docking scores with the SP protocol of -6.2 (1C14) and -6.5 (1ISZ) were observed for these two structures. An additional docking calculation was run on the 1C14 structure with the cofactor and inhibitor (Triclosan) removed to assess binding to the *apo*-enzyme, this gave a score of -6.1. The final docking calculation was performed on the 1DFI structure which only has the cofactor bound. In this structure a loop in not visible which is observed to close over the active site when an inhibitor is bound (as in 1C14/1ISZ), resulting in a more open active site. When the docking protocol was carried out against 1DFI, with the cofactor present, you see a lower prediction (−5.3) of ligandability, which shows that identifying the most suitable target structure can be important. The PTP1B example is also interesting, five of the protocols examining the primary binding site [28]. There is also a PTP1B structure of a ligand in an allosteric pocket [29] (1T4J) which was targeted in one run, this was the most highly scored of the structures by a small margin (−5.7 against a mean score across the PTP1B structures of−5.2).

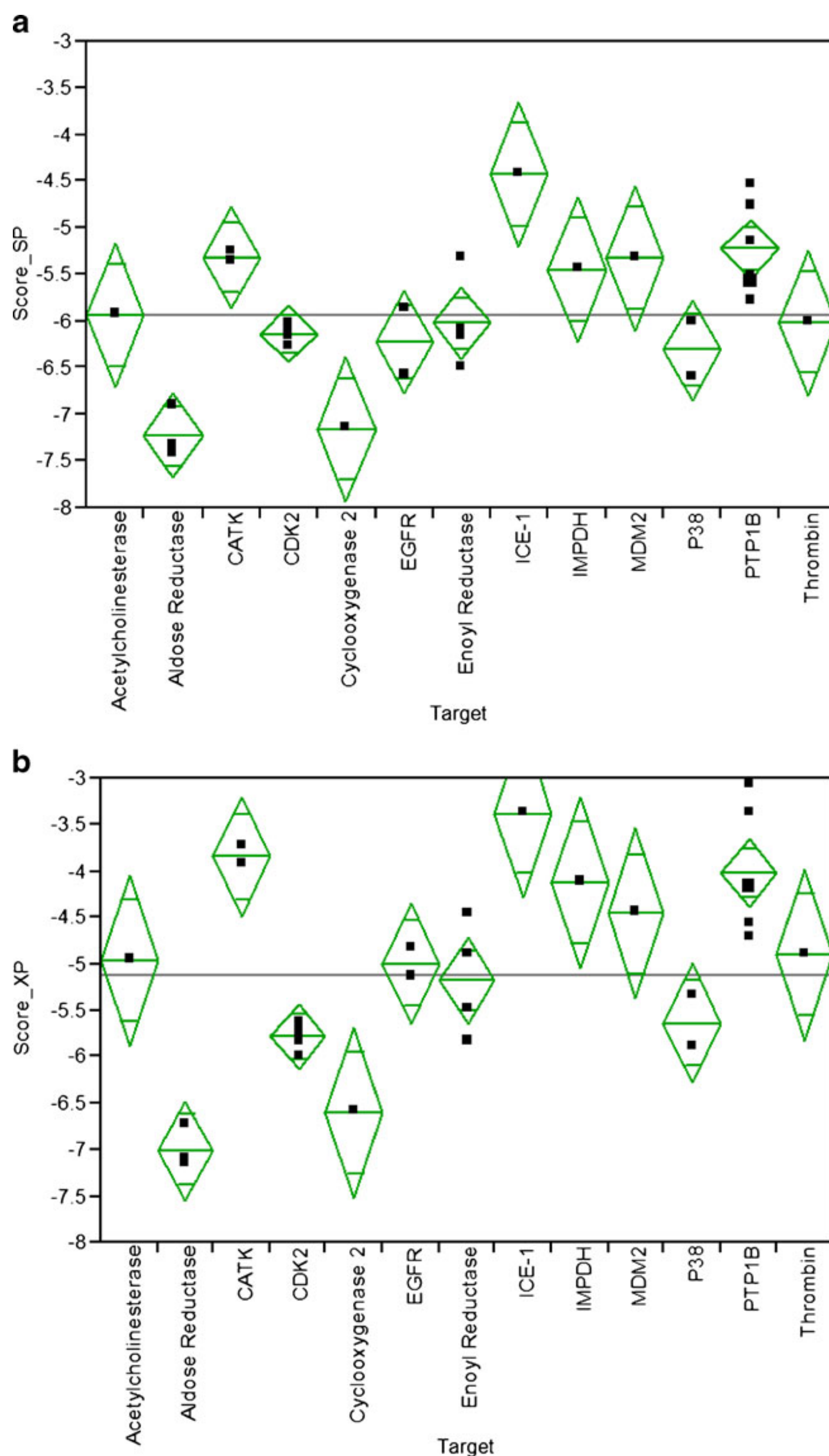

Fig. 5 One-way Anova analysis [25] of the fragment docking scores from different protein structures of a target. The SP protocol data is shown in Fig. 5a and the XP data in Fig. 5b

Assessment of docking approach against target classifications

The following assessment of the druggability of a number of targets has been suggested based on analysis of known inhibitors [8]. The key observation being the potency of the known inhibitors which is used to indicate the tractability of a target. Clearly for more novel targets, data on known inhibitors can be less abundant. The docking scores for the SP and XP protocols were compared against these defined categories of 'druggable', 'undruggable' and 'prodrug/transporter'. Again, the mean score of the fragments against the specified target was used to initially assess ligandability (Fig. 6).

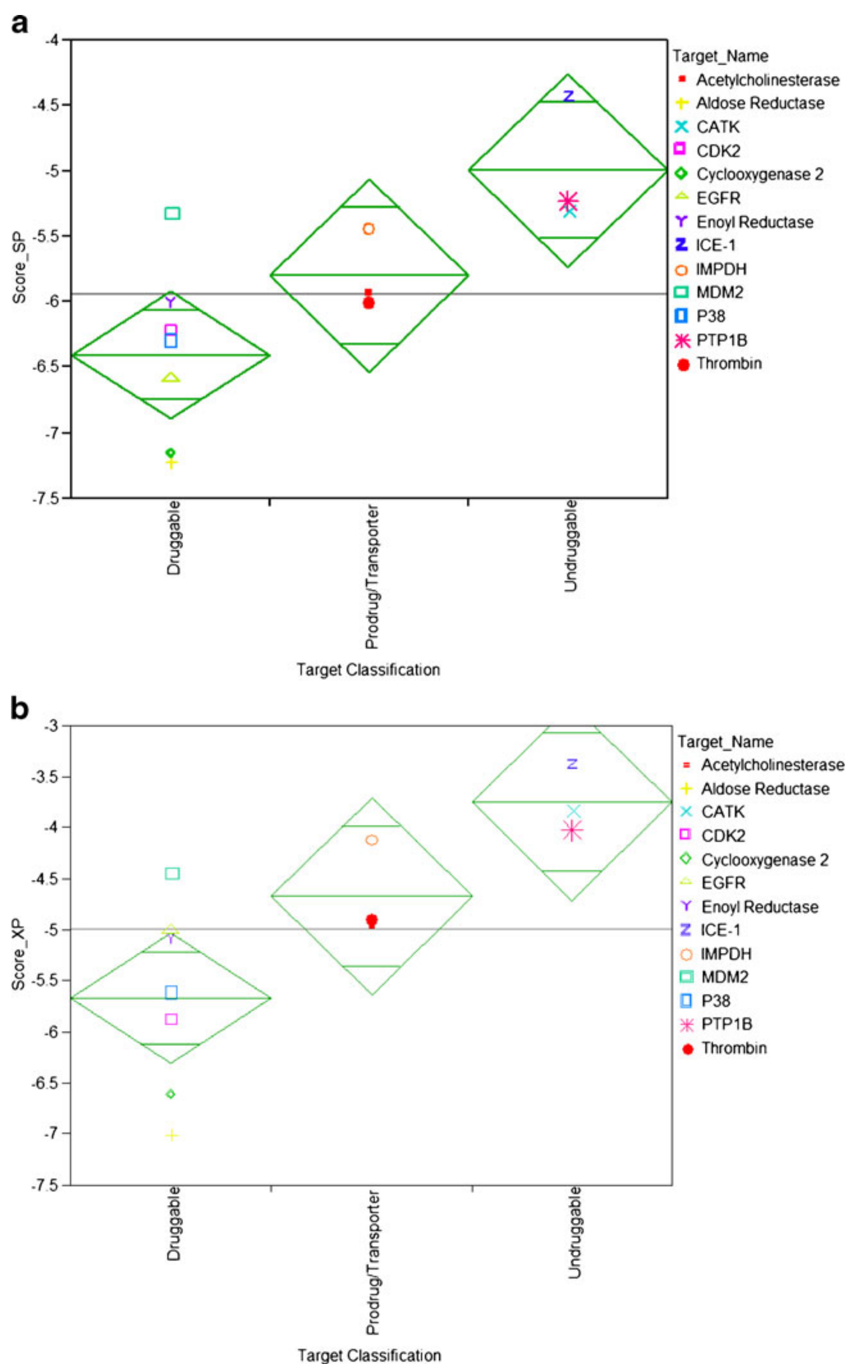

Fig. 6 Comparison of docking scores against target classification using potency of known inhibitors [8]. SP protocol shown in Fig. 6a and XP protocol in Fig. 6b

The docking protocol assessment demonstrated a significant separation between the 'druggable' and 'undruggable' classifications. Using the SP protocol the p-Value=0.0052 and the XP protocol produced p-Value=0.0041 when comparing the separation of these two categories by the ligandability score. However, the 'prodrug/transporter' examples spanned the mid-range of the two categories so were more difficult to differentiate. One outlier from the docking analysis was MDM2, defined as 'druggable' in the target classification. Although inhibitors are known for this target the binding site is challenging protein-protein interface so it is reasonable to expect this target to have a poorer ligandability assessment by docking. Although the 'prodrug/transporter' targets have had potent inhibitors successfully identified, it is expected that they will score less well by ligandability assessments using docking. The reasoning for this is that the active form of the inhibitor usually has a charged (or reactive) warhead which is masked in the prodrug to improve the properties of the compound [30]. As a number of highly charged or reactive

functionalities will have been removed from fragment screening sets then tight binding fragments may not be identified. An alternative fragment set containing more highly charged/reactive functionalities may be required to further probe these targets. However, the fragment set used in this study remains a useful set to assess conventional ligandability and does contain a number of simple acids, bases and other charged groups.

The ligandability prediction from the mean score of all the docked fragments against a target was compared to the prediction using the mean score from just the top 10% ranked fragments against the target. The reasoning behind this being that some tractable targets may have a small number of tight binders which may not be reflected by the mean scores across the full fragment set. However this analysis did not show improved differentiation between the targets, the separation between the druggable and undruggable classes is actually slightly reduced (p-Value=0.0212 for SP protocol and p-Value=0.0944 for XP). The results from the SP and XP docking protocols are shown in Fig. 7.

An alternative way of expressing the ligandability data is to plot the docked scores of the ranked fragments of all the

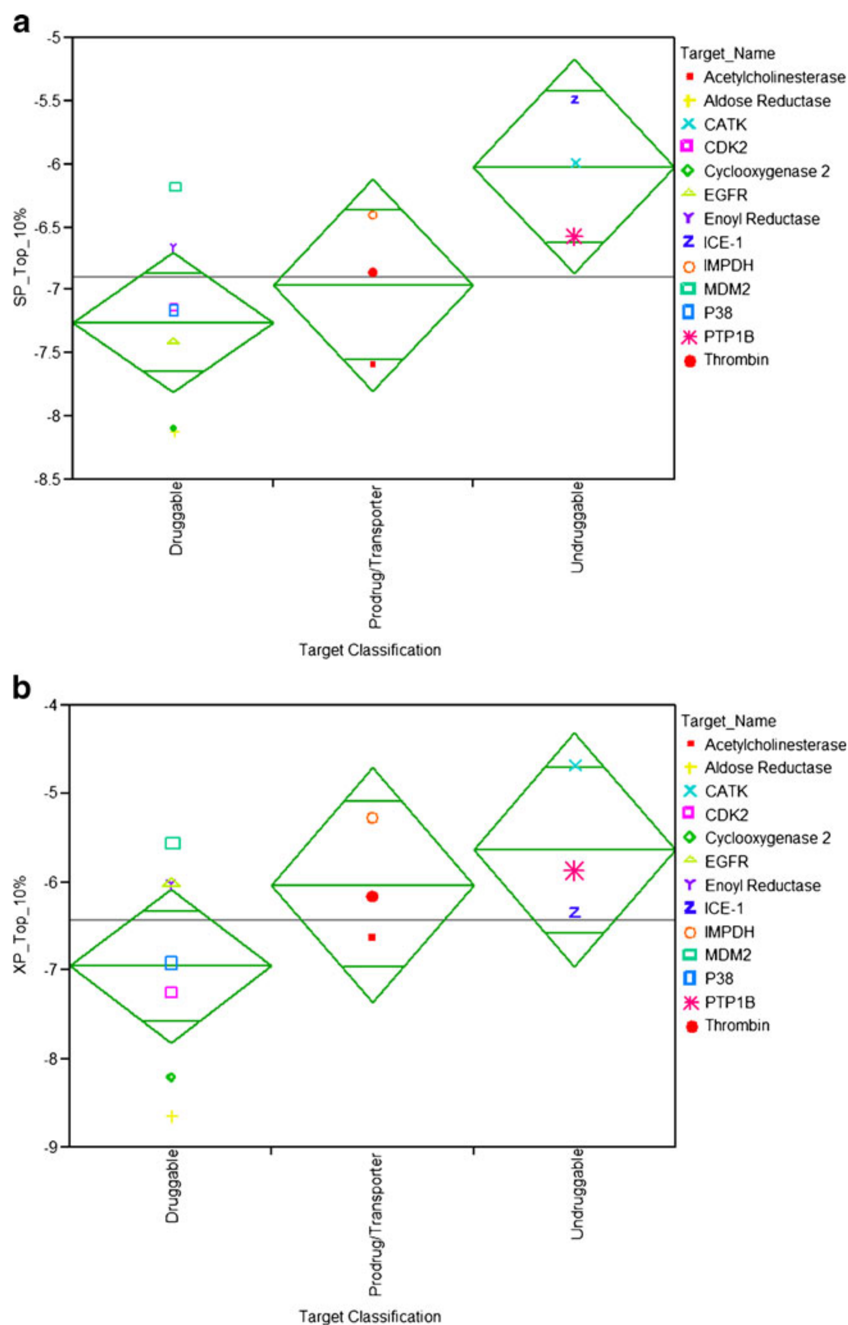

Fig. 7 Comparison of ligandability scores by docking against chemical tractability guided by knowledge of known inhibitors [8]. The mean docking score of the top 10% of fragments against a target defined ligandability in this study. SP protocol shown in Fig. 7a and XP protocol in Fig. 7b

targets on a single plot. This gives a visual way of assessing if some targets have small numbers of fragments with high-predicted affinity or if the range of docking scores for a target has a flatter profile. It may be expected that a flatter profile shows a less interesting pocket than a profile which has a number of fragments predicted with a high affinity. The plot in Fig. 8 supports the early observation that COX-2 and aldose reductase are predicted to have a high ligandability from the docking studies. Thrombin and acetylcholinesterase appear to have a number of highly scoring fragments; these targets are both classified as prodrug/transporter. This may reflect some differences in the difficulty of finding a lead (or fragment hit) compared to being able to modify that lead/fragment into an orally bioavailable drug. For example, it may be possible to identify hits against a certain target but the nature of the binding site may make it difficult to optimize the series whilst remaining in drug-like property space [31].

To provide further information on how targets can be compared two additional scores were utilized. The 'mean score target adjusted' and the 'mean score fragment adjusted'. The mean score targeted adjusted is the ligandability score from the docking of the fragment set (using the mean score of the fragments) subtracted by the mean score of all the targets tested. So a negative number shows a target which has a better predicted ligandability than the mean and vice versa. So clearly the rank ordering of the targets is not affected but it gives a benchmark for the comparison of new targets. As more targets are analyzed using this method then the mean score of all targets will change, especially in the initial stages, so this can be updated. The second score (mean score fragment adjusted) assesses how well each fragment scores to a single target compared to the mean score for that fragment across all targets. So this allows fragments to be identified with a score highly against a certain target, but reduces the score for fragments which score well in multiple targets. The intention being that the impact of the more lipophillic and featureless fragments, which may often act as frequent hitters, do not dominate the ligandability assessment. Therefore this score is compiled by subtracting the mean score of each fragment across all targets from the score against a specific target. The mean of this corrected score for fragments against a target is now used as a prediction of ligandability.

Mean score target adjusted	ligandability score of target—mean ligandability score of all targets
Mean score fragment adjusted	Mean (docking score of fragment against target—mean docking score of fragment against all targets)

These two scores are plotted in Fig. 9 for the assessed targets. SP and XP protocols are shown on the x- and y-axis for both.

The 'mean score target adjusted' allows a clearer comparison against how a studied target compares to the predicted average level of difficulty. Therefore allowing a benchmark against known targets which have previously been the subject of lead generation campaigns. The 'mean score fragment adjusted' does not significantly change the ranking ordering of most of the targets. This suggests that the affect of lipophillic frequent hitters in the assessment by docking is minimal as we aim to remove these from a fragment screening set. This way of scoring the targets could also be misleading if the number of examples from

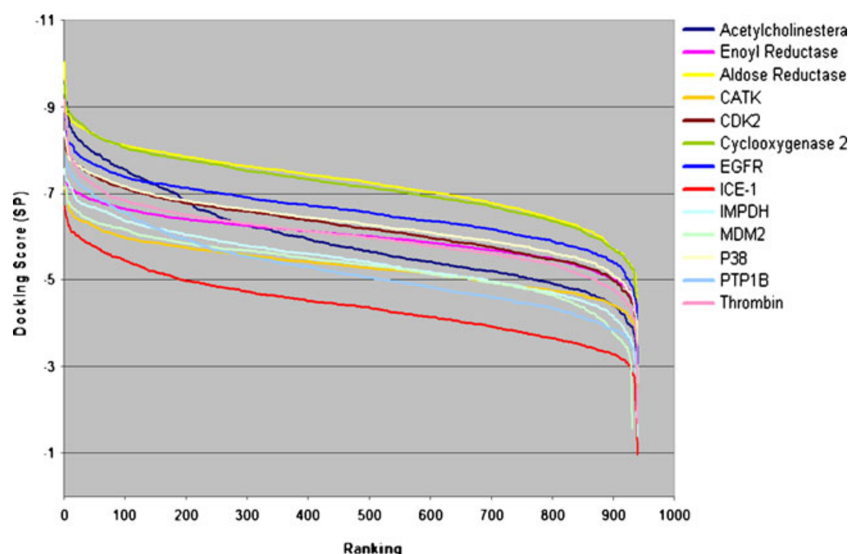

Fig. 8 Profile of the docked fragment scores (SP protocol) for each target in the validation set

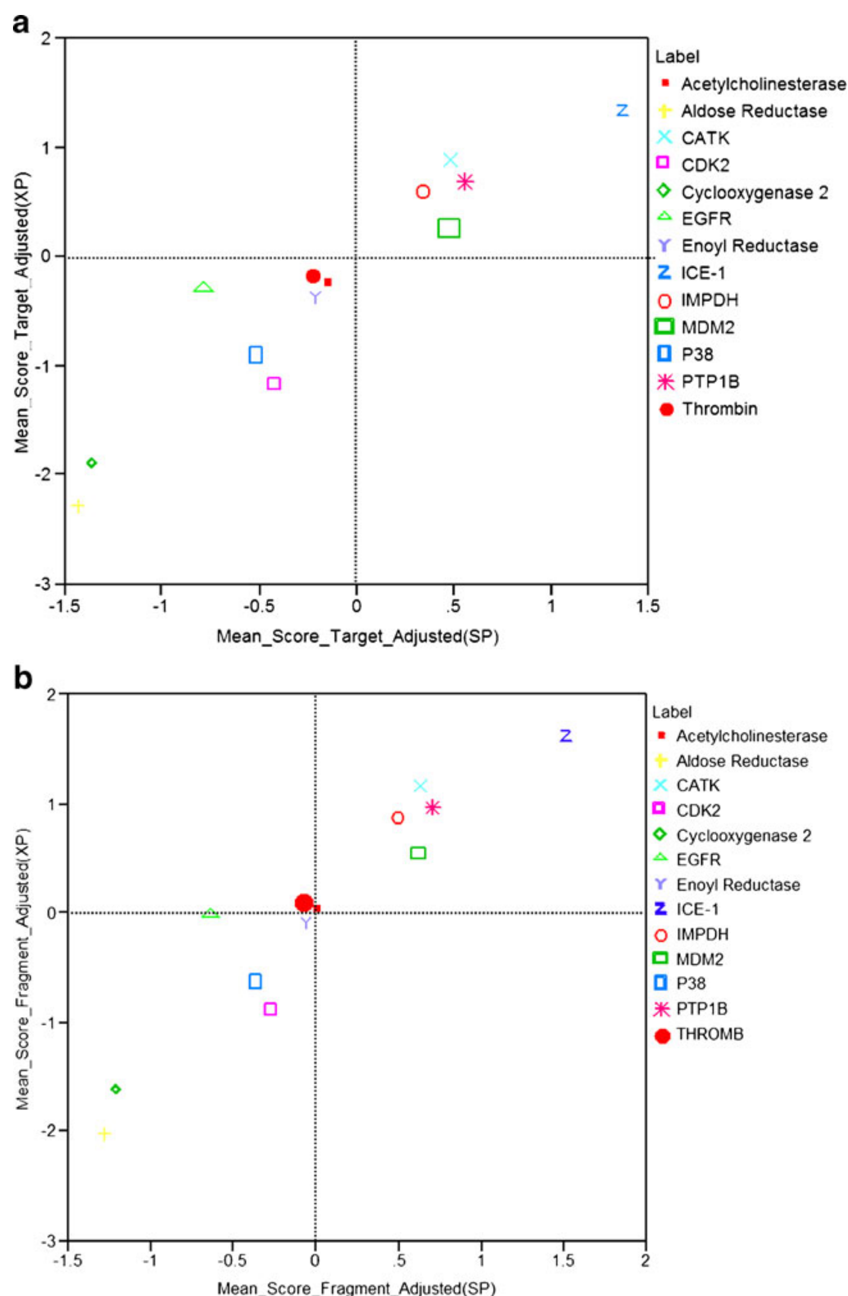

Fig. 9 Comparison of adjusted docking scores to assess ligandability. Fig. 9a shows the 'mean score target adjusted'. Fig. 9b shows the 'mean score fragment adjusted'

different target classes is not well balanced. This could lead compounds from over-represented target classes, such as kinases (CDK2, EGFR, P38 have been analyzed) to be artificially scored lower if similar fragments scored well between these targets. Two further methods of exploiting the docking scores were assessed. One method used a ligand efficiency score (docking score/heavy atoms) but this reduced the separation between the 'druggable' and 'undruggable' categories. Finally the most highly scored docked fragment was used to represent the ligandability of a target but this also failed to improve the separation between 'druggable' and 'undruggable' categories.

Conclusions

The docking method detailed provides an alternative method for assessing the druggability (or ligandability) of target proteins where crystal structures are available. When using the mean docking score for the fragment set against a target the results are largely consistent with the experimental data and approaches reported previously in the literature. Further validation work is to be performed on in-house targets which have already been targeted by lead generation approach in addition to novel enzymes currently under target selection. Where there are a number of targets which could be

prosecuted, of a similar strength of disease linkage, then this information may be able to prioritize targets most likely to return lead-like compounds. This approach may also help to identify the most tractable pocket of a target and may guide the lead generation approach (*i.e.*, virtual/directed screen, HTS, FBLG or a combination of these). Other information will be used in this decision making process, including the knowledge of known ligands/substrates/co-factors of the target and any relevant information or experience around the target class. Clearly the limitation of this approach is the requirement for crystal structures to be available, or where there may be a structure of a close homologue. There is also an additional issue around protein flexibility which suggests that examples which have an allosteric pocket or a significant degree of conformational change in the active site will not be accurately predicted. In addition, for some targets, the primary binding site may not be clearly located so a tool like SiteMap may be required to rank potential binding sites before they are subsequently analyzed. Often, once all these factors and additional information are considered, a high throughput measure to assess large numbers of targets may not be required. However, this approach has shown that a range of targets can be analyzed using this methodology and the more chemically tractable examples can be prioritized in many cases. This approach is to be further evaluated using fragment sets with a broader range of physicochemical properties, this may be particularly valuable for protein-protein interfaces. For druggability assessment using these fragments, computational approaches may have additional value due to the potential issues around the experimental screening of more lipophillic fragments with poor solubility. Another extension of this approach may be the integration of additional methods which may consider protein flexibility. Further work around the binding mode and subsequent affinity predictions of fragments will also be required to increase the predictive power of this methodology.

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