Chapter 14

Prediction and Optimization of Pharmacokinetic and Toxicity Properties of the Ligand

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Abstract

A crucial factor for the approval and success of any drug is how it behaves in the body. Many drugs, however, do not reach the market due to poor efficacy or unacceptable side effects. It is therefore important to take these into consideration early in the drug development process, both in the prioritization of potential hits, and optimization of lead compounds. In silico approaches offer a cost and time-effective approach to rapidly screen and optimize pharmacokinetic and toxicity properties. Here we demonstrate the use of the comprehensive analysis system pkCSM, to allow early identification of potential problems, prioritization of hits, and optimization of leads.

Key words ADMET predictions, Computational medicinal chemistry, Drug development, Hit prioritization, Lead optimization, Pharmacokinetics, Toxicity

1 Introduction

Drug development is a fine balance of optimizing drug like properties to maximize efficacy, safety, and pharmacokinetics, with the ultimate goal being to ensure that it can reach the target site in sufficient concentrations to produce the physiological effect safely. Getting this balance right is essential for the successful introduction into the clinic.

The pharmacokinetic profile of a compound defines its absorption, distribution, metabolism, and excretion (ADME) properties, while toxicity describes a compound's safety profile. Small structural modifications can significantly affect the pharmacokinetic and toxicity properties of drug candidates.

Experimental evaluation of small-molecule pharmacokinetic and toxicity properties is both time-consuming and expensive and does not always scale reliably between animal models and humans. To address this, many computational approaches have been developed to guide compound design and selection throughout the drug development process (Fig. 1). These rely upon associations

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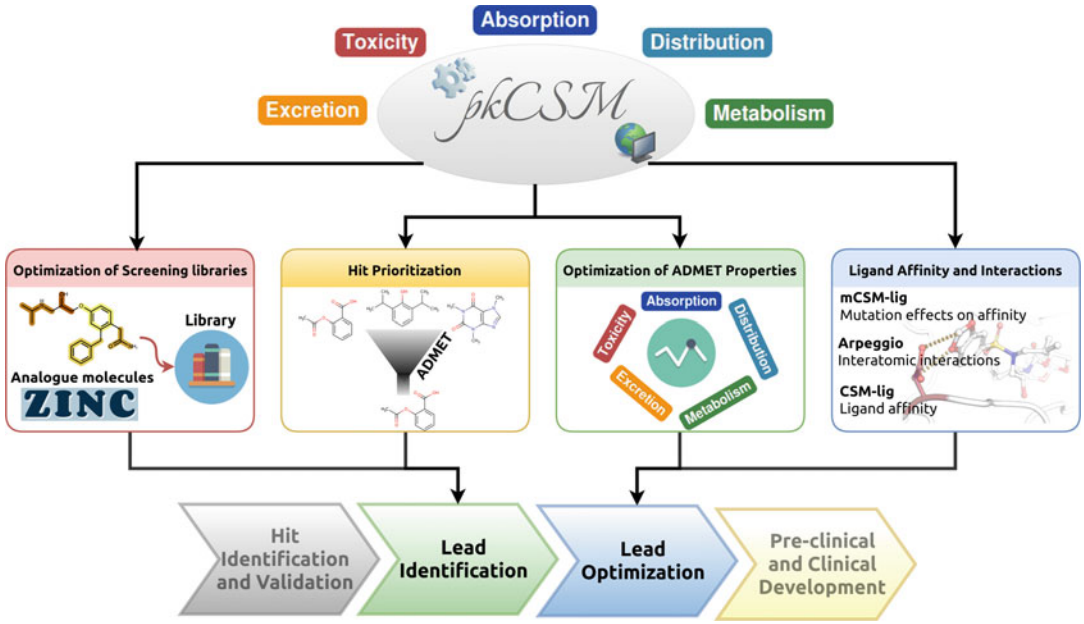

Fig. 1 Screening compound pharmacokinetic and toxicity properties throughout the drug development process using pkCSM as a way to guide and facilitate the drug design process, minimizing risks of failure due to poor ADMET

between the chemical structure of a compound of interest and experimental data for similar structures, and include data-based approaches such as 2D and 3D quantitative structure–activity relationship [1–3], similarity searches [4, 5], and structural-based methods such as ligand–protein docking [6, 7] and pharmacophore modeling [8]. While many of these are unfortunately not freely available, the recent development of pkCSM [9] (http://structure.bioc.cam.ac.uk/pkcsm) has provided a new freely available tool to comprehensively characterize the pharmacokinetic and toxicity properties of your compounds of interest.

pkCSM uses the concept of graph-based structural signatures to study and predict a diverse and complementary range of ADMET properties for novel chemical entities, including the following:

- *Absorption*: Water solubility, Caco2 permeability, human intestinal absorption, and skin permeability, and whether the molecule is a P-glycoprotein substrate or inhibitor.
- *Distribution*: Human volume of distribution, human fraction unbound in plasma, blood–brain barrier and central nervous system permeability.
- *Metabolism*: Whether the molecule is a Cytochrome P450 substrate or inhibitor.

- *Excretion*: Total clearance and whether the molecule is a renal OCT2 substrate.
- *Toxicity*: AMES toxicity, human maximum tolerated dose, oral rat acute and chronic toxicity, hERG inhibition, hepatotoxicity, and skin sensitization.

2 Materials

2.1 Running pkCSM

1. List of compound structures of interest formated as canonical SMILES:
   (a) A SMILES string is a widely used line notation for representing the atomic composition and structure of chemical entities. In short, a SMILES can be generated from a graph representation of a molecule (atoms as nodes and bonds as edges) by executing a depth-first search, generating a spanning tree. Several different SMILES strings can be generated for the same molecule, depending on the search algorithm used. Several algorithms, however, have been developed to generate the SMILES for a given compound in a unique way (Canonical SMILES). Users are advised to use the OpenEye Canonical SMILES as syntax noncompliant molecules might not be processed correctly. Several SMILES strings can be combined into a file for submission to the pkCSM platform to analyse multiple structures at once.
   (b) Molecules of interest can be converted from and to the SMILES format using any of several different open source libraries currently available, including Open Babel [10] and RDKit. There are also several online resources that can generate smiles (e.g., https://cactus.nci.nih.gov/translate/).

2.2 Running CSM-Lig, Arpeggio and mCSM-Lig

Any valid Protein Data Bank (PDB) files are acceptable for running the servers as long as they comply with the format, as defined in http://www.wwpdb.org/documentation/file-format. This way, the servers are capable of handling crystallographic structures as well those generated via molecular docking and homology modeling [11–13] (*see* **Notes 1** and **2**).

1. CSM-lig:
   (a) Structure of the compound bound to the protein target in PDB format; when no experimental structure of the complex is available, molecular docking can be used to model the complex.
   (b) Ligand information;
   - Ligand three-letter code (as used in the PDB file);

- SMILES string of the ligand bound/docked.

2. mCSM-lig:
   (a) Structure of the compound bound to the protein target in PDB format;
   (b) Mutation information, including:
   - The mutation code, composed by one-letter code of the wild-type residue, residue position, and one-letter code of the mutant residue (e.g., D30N);
   - The chain ID of the wild-type residues;
   - Ligand three-letter code (as used in the PDB file).

   (c) Wild-type affinity in nM. This only needs to be approximate. Experimental data for many molecules can be found in the BRENDA database [14]. Alternatively, the predicted affinity from CSM-lig [7] can be used.
3. Arpeggio:
   (a) Structure of the compound bound to the protein target in PDB format.
   (b) To calculate and visualize interactions being made by the compound, the ligand can be selected from the list of heteroatom groups. Alternatively, the ligand can be specified in the format "/a/b/", where a denotes the chain ID and the compound number, as used in the PDB file. Example: /A/30/will select ligand number 30 of chain A.

3 Methods

3.1 Running pkCSM

1. Open up the pkCSM prediction server on a browser (pkCSM is compatible with most Operating Systems and browsers. We, however, recommend using Google Chrome): http://structure.bioc.cam.ac.uk/pkcsm/prediction;
2. Provide either an input file with a list of molecules in SMILES format (up to a maximum of 100 molecules) or supply a single SMILES string for an individual molecule (Fig. 2a) (*see* **Notes 3** and **4**)
3. Choose the prediction mode, selecting either between the individual ADMET property classes (**A**bsorption, **D**istribution, **M**etabolism, **E**xcretion, and **T**oxicity) by clicking on their corresponding button, or run a systematic evaluation of all predictive models.
4. For single molecules (Fig. 2b), the predictions will be displayed in tabular format, along with a list of calculated molecular properties. The information shown include the ADMET property being predicted, the predictive model name, the actual

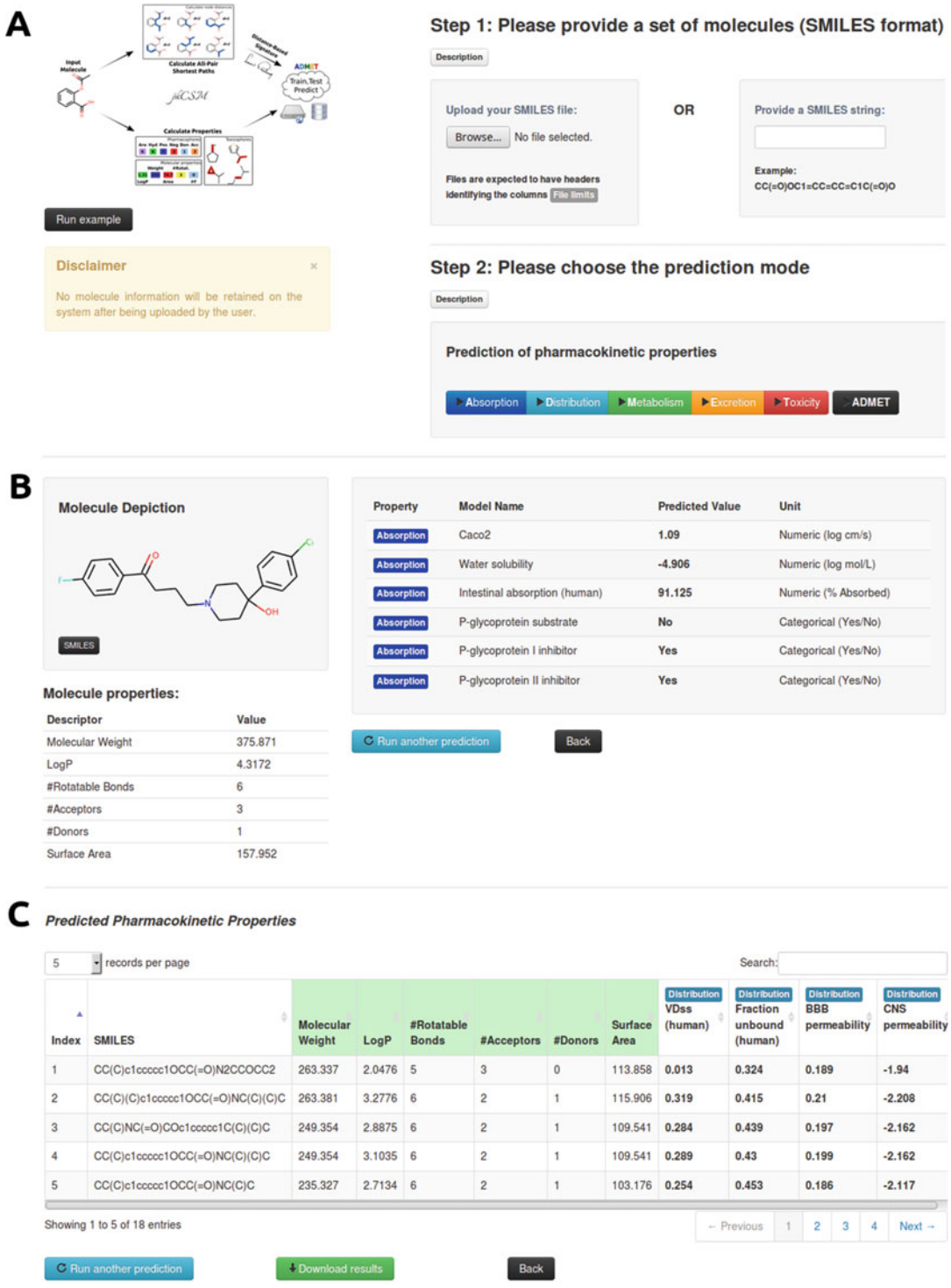

Fig. 2 pkCSM web interface. (**a**) depicts the submission page from pkCSM where users can submit either a single or list of compounds as canonical SMILES to predict their pharmacokinetic and toxicity properties by clicking in the corresponding buttons. A button for calculation of all ADMET properties is also available.

predicted value and whether the prediction is numerical, indicating the unit of the predicted value, or categorical. A depiction of the molecule is also shown.

5. Predictions for multiple molecules will be shown in an interactive tabular format that can be downloaded as a CSV file (Fig. 2c). Users have the option to sort the table by any column and search/filter specific compounds.

3.2 Interpretation of Output

1. Additional information about the predictive models and how to interpret the pkCSM predictions can be found via the Theory menu of the web server at: http://structure.bioc.cam.ac.uk/pkcsm/theory.
2. The five more critical pharmacokinetic parameters are described below.
   (a) *Plasma half life*—This is the time required for the plasma concentration of a drug to decrease by 50%. It can be calculated from the natural log of the ratio of volume of distribution and clearance.
   (b) *Oral bioavailability*—This is the fraction of a drug that reaches systemic circulation after oral dosing. One of the crucial steps of this is a compound's ability to be absorbed through the intestine. pkCSM provides two predictive measures of this—Caco-2 permeability and human intestinal absorption.
   (c) *Plasma protein binding*—Most drugs in plasma will exist in equilibrium between an unbound state, or bound to serum proteins. The efficacy of a given drug may be affected by the degree to which it binds proteins in blood, as the more that is bound the less efficiently it can traverse cellular membranes or diffuse. This can affect renal excretion, blood–brain barrier permeability, and interactions with the target of interest. Hydrophobic compounds often will bind nonspecifically to many hydrophobic sites on many proteins. High-throughput screening often identifies hydrophobic hits, which can be extremely difficult to optimize. Conversely, engineering plasma protein binding has been used to improve the half-life of peptides by reducing renal excretion. pkCSM predicts the fraction of a drug that will remain unbound, based upon human data.

Fig. 2 (continued) (**b**) shows the result page for the predictions of *Absorption* properties for a single molecule. The molecular properties of the ligand are shown on the left hand side of the screen. (**c**) shows the results page for the prediction of *Distribution* properties for multiple molecules. The results can be downloaded as a tab-separated file

(d) *Volume of distribution*—The volume of distribution is the theoretical volume that the total dose of a drug would need to be uniformly distributed across to give the same concentration as in blood plasma. The higher this number, the more the drug distributed in tissues as opposed to plasma. Hydrophilic and negatively charged compounds often have small volumes of distribution, as they do not diffuse effectively into tissues. Compounds that are mostly bound to plasma proteins will also appear to have a small volume of distribution. Hydrophobic and positively charged compounds often have large volumes of distribution as they can readily dissolve in and interact with the negatively charged cell membrane. pkCSM predicts the logarithm of the steady state volume of distribution based upon human clinical data. The ideal volume of distribution depends upon the disease being treated and the targeted half-life. For example, often a large tissue distribution, corresponding to a large volume of distribution is often considered desirable for antibiotics and antivirals targeting intracellular pathogens. By contrast, compounds with a small volume of distribution enable better control of drug plasma levels, important for compounds with small therapeutic windows. Distribution, targeting and clearance of small molecules can also be altered through the use of drug carriers [15].

(e) *Clearance*—This is the rate at which plasma is cleared of the drug. Drug clearance occurs primarily as a combination of hepatic clearance (metabolism in the liver and biliary clearance) and renal clearance (excretion via the kidneys). It is related to bioavailability, and is important for determining dosing rates to achieve steady-state concentrations. pkCSM predicts the total clearance of a drug based upon data from humans.

3. Toxicity measurements are important to consider relative to the concentration of a compound needed to exert a therapeutic effect. This is known as the Therapeutic Index/Window—the ratio of the dose that leads to lethality in 50% of the population (Rat LD50 in pkCSM) divided by the minimum effective dose for 50% of the population. Larger therapeutic indices are preferable since a much larger dose of a drug would need to be administered to reach the toxicity threshold than that needed to elicit the therapeutic effect.

3.3 ADMET Optimization of Screening Libraries

1. When developing a screening library, or identifying analogs to screen, for a particular condition, it is worth tailoring it in order to enrich it for compounds with more favorable properties.
2. Identifying analogues to screen can help expand and develop the initial hit. This is often performed through 2D and 3D

similarity searches of initial hits using databases of compounds from your commercial suppliers (analoging by cataloging) or large databases (such as the ZINC database: http://zinc.docking.org/search/structure).

3. Compounds should be screened for potential problems including PAINS groups [16], mutagenic groups and groups with known toxicity issues.
4. While maintaining broad chemical diversity, the library can be screened through pkCSM and used to enrich particular ADMET features favorable for the target protein/disease (e.g., BBB permeability for neuroactive compounds [12, 17–19]).

3.4 Modifications to Improve ADMET Properties

1. pkCSM predictions can be used when composing screening libraries, enriching them with compounds that suit the drug target. For example, when screening for neuroactive compounds, it would make sense to enrich your screening libraries for compounds with high blood–brain barrier and central nervous system permeability.
2. However, when a lead compound has been identified, there are chemical modifications that can be performed which may improve the pharmacokinetic and toxicity profile. Small structural modifications can significantly affect the pharmacokinetic and toxicity properties of drug candidates.
3. Using the multiple molecule prediction mode of pkCSM, large libraries of analogues can be screened to identify compounds with promising ADMET profiles. A few common medicinal chemistry strategies used to improve pharmacokinetic profiles are described below. It is always worth bearing in mind how any proposed alterations might affect how the compound binds to the target of interest. While sometimes a successful strategy, there are many times when new cores will need to be explored in order to move away from these unfavorable properties.
   (a) *Improving oral bioavailability*: Oral bioavailability is a function of the proportion of a drug absorbed through the intestine, and the amount that is metabolized in the liver before entering the systemic circulation. Passive intestinal absorption correlates with size, with absorption decreasing as molecules polar surface area increases beyond 60 A^2, with negligible absorption observed beyond 140 A^2. Charged and hydrophilic compounds absorb best when their molecular weight is below 200 Da, and hydrophobic compounds need to be at least partially water soluble.
   (b) *Improving metabolism profile*: High levels of cytochrome P450 metabolism will reduce oral bioavailability and plasma half life [20]. This can be reduced through altering the logP and PSA, and by blocking hydroxylation through

fluorination and introduction of heteroatoms at potential sites of hydroxylation.

Other forms of metabolism to watch out for include metabolism by alcohol dehydrogenase, oxidases and reductases, esterases, phosphatases, and proteases; and adduction by strong nucleophiles including glutathione. Alternatively, sometimes metabolic inhibitors can be used to potentiate drug action [21, 22].

(c) *Improving excretion profile*: High levels of renal excretion will lead to lower plasma half lives. Increased levels of protein plasma binding and volume of distribution can reduce renal excretion. Some charged molecules may be actively secreted. Neutral and lipophilic compounds may be resorbed back into plasma.

(d) *Improving permeability*: Blood–brain barrier permeability is linked to small (below 600 Da, polar surface area below 40 A^2), uncharged, lipophilic compounds (log*P* above 0) with few rotatable bonds. Groups that form hydrogen bonds reduce blood–brain barrier permeability. Blood–-brain barrier permeability may also be decreased through active excretion by P-gp transporters.

(e) *Avoiding toxicity*: Currently, along with lack of efficacy, toxicity issues are the main reason for drug failure. Similar to how the incorporation of ADME screening into the early drug development pipeline drastically reduced failures (in the 80s and 90s pharmacokinetic failures were a leading cause of drug failures), consideration of toxicity issues early in the drug development process can mitigate these issues. Strong electrophiles, and functional groups that are prone to the formation of strong electrophilic metabolites, are often toxic and/or mutagenic. Chromophores such as quinolines may be phototoxic and lead to skin sensitization. Inhibition of human Ether-a-go-go related gene has been linked to the withdrawal of several drugs that led to cardiac complications, and should be avoided.

3.5 Identification of Changes to Affinity

1. Any changes to the drug need to be considered with respect to how they may alter binding to the target. Using a structure of the compounds with the target, these effects can be explored in different ways.
2. *Calculating interatomic interactions between protein and ligand*: A map of important molecular interactions being made by a compound can be generated and visualized using the Arpeggio webserver [23] (http://structure.bioc.cam.ac.uk/arpeggio/). Figure 3a shows the Arpeggio's results pages.

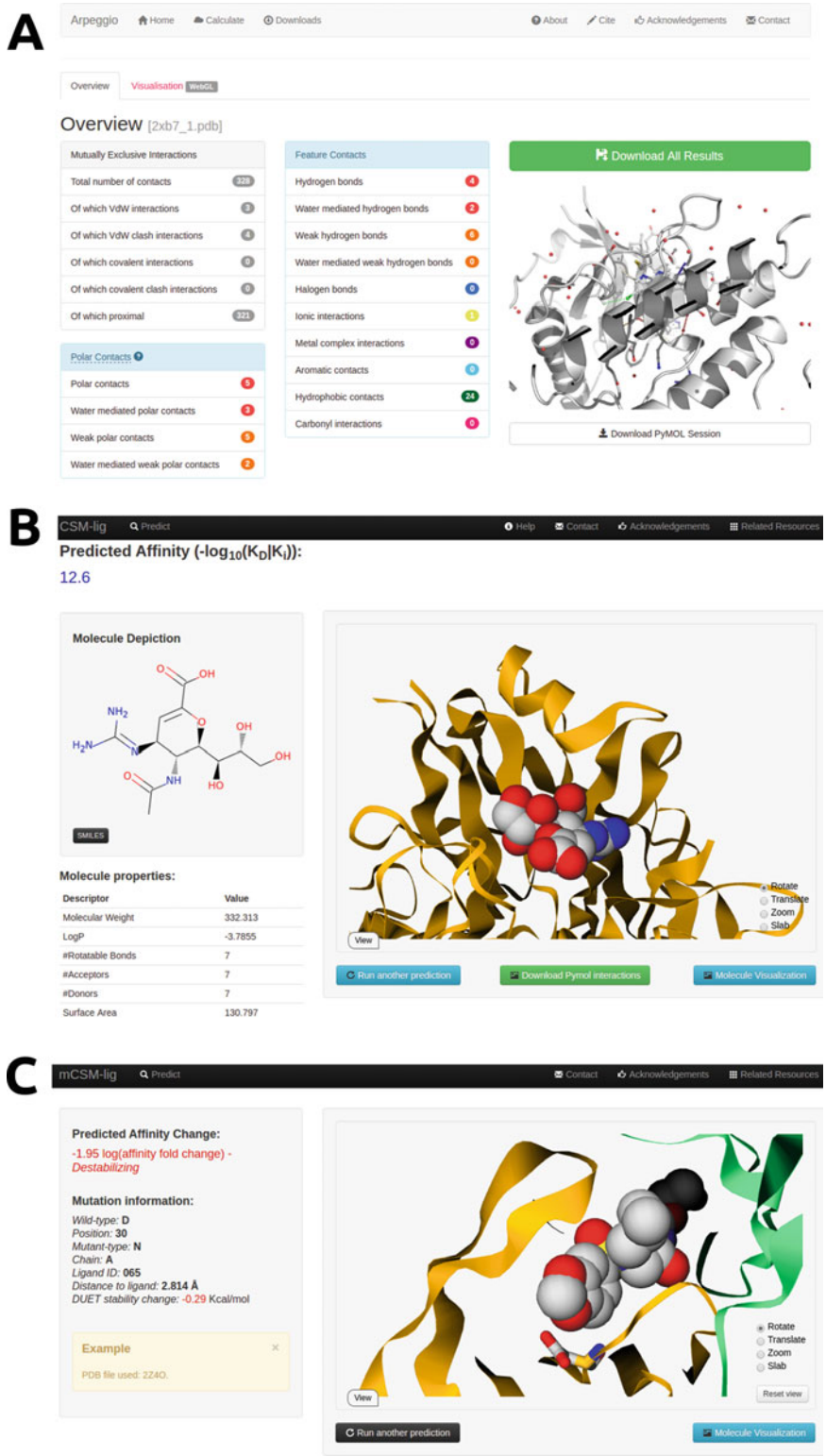

Fig. 3 Assessing different aspects that influence protein–ligand affinity. (**a**) depicts the result page for Arpeggio. A color-coded list of identified interactions and their number is exhibited. (**b**) shows the result

Users have the option to download a Pymol session file to visualize the interactions calculated (*see* **Note 5**).

3. *Calculating protein–ligand affinity and assessing docking poses*: While docking scores have been considered unreliable, a range of new approaches are providing more accurate estimations of binding affinity. For example, the binding affinity of modified compounds can be predicted using CSM-lig [7] (http://structure.bioc.cam.ac.uk/csm_lig/). Figure 3b shows CSM-lig results page. Users have the option to asses either a single protein–ligand complex or submit a compressed file with multiple poses (limited to 50 MB in size) (*see* **Note 6**).
4. *Calculating effects of mutations and identifying resistance mutations*: Potential resistance mutations can be identified using mCSM-lig [24–26] (http://structure.bioc.cam.ac.uk/mcsm_lig/). This can be used to help identify likely resistance mutations early in the drug development process [27], in order to minimize interactions with these resistance hot-spots. When considering possible resistance mutations it is important to consider other affects the mutation might have upon protein stability [28–31] and other interactions [24, 28, 32–37]. The mCSM-lig results page is shown in Fig. 3c (*see* **Note 7**).

4 Notes

1. When uploading a PDB structure generated via homology modeling or docking, make sure a valid chain ID is present. The servers will not accept white spaces as valid chain IDs. You can renumber the chain using a text editor, pymol or web servers (http://www.canoz.com/sdh/renamepdbchain.pl).
2. When using NMR solved structures, it is a good practice to select a single model to be submitted (even though the servers will automatically select the first model).
3. If your compound will not run on pkCSM, make sure that you are using Canonical SMILES.
4. When uploading a file for the servers (e.g., a list of SMILES for pkCSM, a list of mutations for mCSM-lig) make sure that you

Fig. 3 (continued) page for CSM-lig. A Pymol session with calculated interactions is available, as well as the calculated ligand properties and its molecule depiction. The predictions are given as the -log(K_D or K_i). (**c**) shows the result page for mCSM-lig. The mutation information is shown and the prediction is given as the log(affinity fold change). Negative values, which will be colored in red, denote mutations reducing ligand affinity

upload a purely textual file, as other formats will now be recognized (e.g., .doc, .xls, and others).

5. If your protein will not run on Arpeggio, mCSM-lig or CSM-lig it is worth checking the PDB structure for nonstandard entities, including:
   - Nonstandard atom groups (e.g., metal atoms such as zinc in capitals ZN);
   - Nonstandard residues;
6. Other possible causes of error while running servers that rely on protein–ligand complexes include:
   (a) Ligand is missing from the structure;
   (b) Ligand information (ID/number/chain) does not match the provided PDB file;
   (c) In the case of mCSM-lig, mutation information is not compatible with PDB file (wild-type residue could not be found in the provided position/chain).
7. Structures with multiple ligands bound might interfere with the predictions (especially if they are in close proximity to the ligand or mutation of interest) since they will be taken into consideration in the calculations.

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