Molecular Modeling to Predict and Understand Chemical Toxicity

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research&development

Science Question

There is a paucity of relevant experimental information available for the evaluation of the potential health and environmental effects of many man made chemicals. Therefore, there is a compelling need to develop information that would enable the screening of the potential health and environmental effects of large numbers of man-made chemicals. Knowledge of the potential pathways for activity provides a rational basis for the extrapolations inherent in the preliminary evaluation of risk and the establishment of priorities for obtaining missing data for environmental chemicals. The differential step in many mechanisms of toxicity may be generalized as the interaction between a small molecule (a potential toxicant) and one or more macromolecular targets. The small molecule may be the chemical itself or one of its descendents. An approach based on computation of the interaction between a potential molecular toxicant and a library of macromolecular targets for toxicity (The Toxicant-Target approach) has been proposed as an element for chemical screening and testing prioritization. In order to use a library of this type, a rapid method to evaluate interactions between the small molecule and a (macromolecular) target is needed. Molecular "docking" has been developed to screen large chemical libraries for molecules that interact strongly with specific sites on proteins and therefore are potential pharmaceutical agents. This approach has infrequently been applied to investigate the potential activity of weaker agents.

Can "docking" and other molecular modeling approaches be applied to screen for chemicals that interact with a macromolecular target? Can the results of this approach be used in conjunction with experimental assays in a screen for potential toxicity? Using experimental results for the rat estrogen receptor, will "docking" separate agents that bind weakly from inactive chemicals? For any chemical screening approach that depends on a data base and molecular parameters, how is its range of applicability determined?

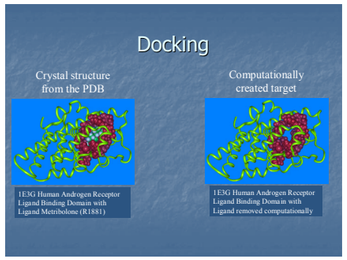
Research Goals

1. To develop an approach for applying "docking" and other molecular modeling methods to problems of screening and prioritizing chemicals for potential toxicity.
2. To test two "docking" methods for their capacity to identify chemicals that compete weakly with E2 for the active site of the estrogen receptor.
3. To develop methods for determining the domain of applicability of any relationship that predicts chemical toxicity from other molecular parameters (experimental or computational).

Methods/Approach

Structurally Based Computational Screening

Potential macromolecular targets for toxicity are DNA and protein. Docking approaches have been used to screen large chemical libraries for potential pharmaceutical agents. The targets used have been almost exclusively protein but there is no reason why a similar approach could not be used for molecules that interact with DNA.



First targets are constructed from receptor-ligand crystal structures by computationally removing the ligand. If the data exists multiple targets may be created for the same protein in order to consider protein flexibility or multiple binding modes.

The potential ligands are then introduced into the computational target and the most stable toxicant-target poses identified using two different approaches.

1. Considering the entire molecule as flexible (FRED)
2. Decomposing the molecule into substructures (eHiTS)
   a. Docking each substructure separately
   b. Recombining the substructures in the target

The energy of each potential ligand-target pose is computed from heuristic functions fitted to reproduce a large number of known protein-ligand structures. These functions are not adjusted for each specific protein or class of potential toxicants.

This approach has been successfully applied to aid in the discovery of novel pharmaceutical agents (strong binders). However, it has not often been used to separate potential weak binders from nonbinders, more like the problem of screening environmental chemicals.

A Data Set

A library of 281 environmentally relevant chemicals was tested in the same laboratory with the same protocol for their capacity to compete with radiolabeled 17-β-estradiol for their binding to the rat ER. The advantages of comparing computational molecular "docking" results to the experimental results for this library are:

First, there are a number of excellent crystal structures of both α and β estrogen receptors available in the Protein Data Bank that can be used to synthesize macromolecular targets for computer "docking".

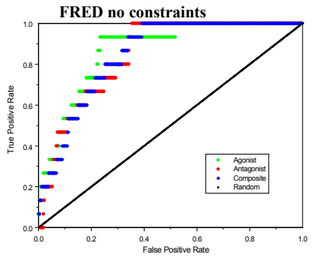
Second, the data set is mostly inactive chemicals. Only 15 chemicals were found to be active and most only weakly so.

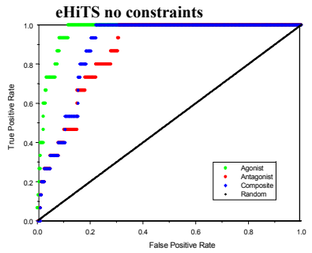
Third, the experiments yield a relatively direct measurement of what is modeled in computational "docking", the energy of interaction between the test chemicals and the receptor compared to the energy of interaction of the receptor with 17-β-estradiol.

Targets

Four targets have been created. Targets for both ERα and ERβ with both agonists and antagonists bound were made from crystal structures. The experimental tissue preparation contains primarily ERα and methods that combine results for docking into ERα and ERβ did not inprove results compared to just ERα. Docking results for discriminating between active and inactive molecules are shown below.

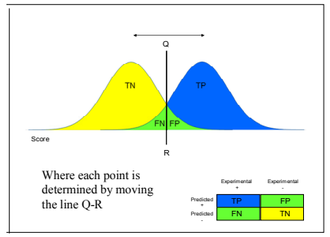
Results/Conclusions





A Summary of the results for each approach without constraints applied.

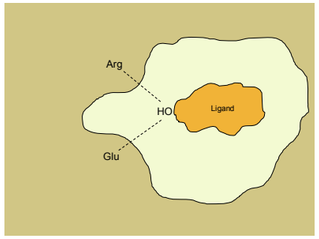
True Positives Identified	5	10	14	15
Fred agonist	16	51	76 (27)	153 (54)
Fred-antag	20	57	105	109 (39)
Fred- composite	23	59	104	119 (42)
eHiTS agonist	8	18	36	46 (16)
eHiTS antag	18	50	95	97 (35)
eHiTS composite	18	51	68	74 (26)

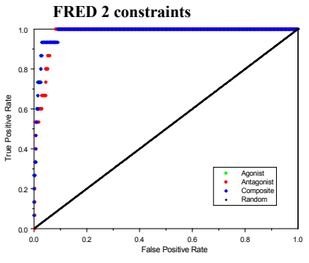


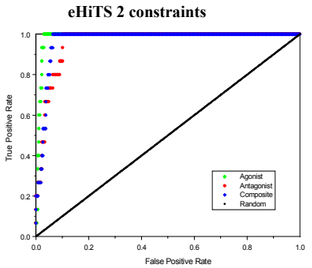
The results from computational "docking" are scores (a surrogate for the interaction energy) for each potential ligand-protein pair**.** Using eHiTS all active chemicals are found by the agonist target in the first 16% of the chemicals. Using FRED 14 of the 15 active chemicals are found by the agonist target in the first 27% of the chemicals. The 15th chemical is found best by the antagonist target.

For the preceding results the best score for each chemical was chosen without consideration of the geometry of binding between the toxicant and the target. A pharmacophore for binding to the estrogen receptor has been developed. A simplification of this pharmacophore is used as a constraint on the allowed toxicant-target poses for the next set of results. For eHiTS, all active chemicals are found by the agonist target in the first 8% of the chemicals. (There are only 8 false positives.) For FRED, the first 14 chemicals are found in 8% of the chemicals but it has more difficulty finding the 15th chemical.

A simplified Pharmacophore







A Summary of the results for each approach with 2 constraints applied.

True Positives Identified	5	10	14	15
Fred agonist	6	14	22 (8)	39 (14)
Fred-antag	6	18	30	37 (13)
Fred- composite	7	15	29	48 (17)
eHiTS agonist	7	14	20	23 (8)
eHiTS antag	10	19	41	42 (15)
eHiTS composite	10	20	29	32 (11)

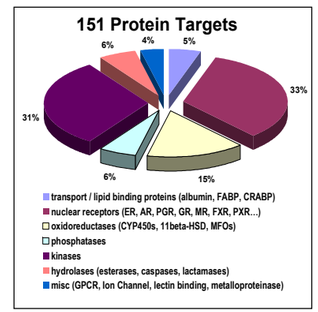
Impact and Outcomes

It has been demonstrated that an approach developed for enriching chemical libraries for likely candidate pharmaceuticals (strongly active molecules) is also capable of separating weakly active chemicals from inactive ones. The relative energies for chemicals interacting at specific targets for toxicity may be used by themselves or in conjunction with other parameters to predict chemical toxicity or prioritize chemicals for further testing. This approach may be used for targets where crystal structures are available and also for targets similar to macromolecules where the structures are known.

Future Directions

The Toxcast chemicals have been docked in 150 targets. Pharmacophores for as many of the targets as possible are being developed and the docking poses will be filtered by the pharmacophores.

The evaluation of the capability of computational methods to predict toxicity or any multi-parameter method for chemical screening, requires an understanding of the position of an untested chemical in chemical and biological space. A method is being developed for evaluating the domain of applicability for any multiparameter method of this type.



References

Rabinowitz, JR, Little, SB, Laws, SC, and Goldsmith, M-R (2009) Molecular Modeling for Screening Environmental Chemicals for Estrogenicity: Use of the Toxicant-Target Approach. *Chem. Res. in Tox*, in press

Rabinowitz, JR, Goldsmith, M-R, Little, SB, and Pasquinelli, MA. (2008) Computational Molecular Modeling for Evaluating the Toxicity of Environmental Chemicals: Prioritizing Bioassay Requirements. *Environmental Health Perspectives*, 116, 573-577.