Application of the *In Silico* Toxicant-Target Approach to Screening a Chemical Library for Estrogenicity

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research&development

Abstract

In many mechanisms for the adverse effects of environmental chemicals a critical and perhaps differential step requires the interaction of the chemical with a biological macromolecule. Where the macromolecular target is a receptor, this interaction may be studied by computationally docking the putative ligand into the receptor binding site. Environmental estrogenicity is an example of a process that can be modeled by this *in silico* approach. In this study the capacity of a series of 318 chemicals to bind to the estrogen receptor has been evaluated using two different methods for docking. Each method depends on semi-empirical approaches to evaluate the interaction but varies in these specifics: 1) The method for the discovery of the best possible fit between the putative ligand and the receptor, 2) the determination of the energetics of each fit, 3) the semi-empirical atom based parameterization of the interaction. The data set studied contains 281 chemicals recently evaluated using a single rat uterine ER binding assay. This data set contains chemicals that bind much more weakly than estrogen and non-binders. In addition, 37 known strong binders were added. The protein targets were derived from known rER and hER crystal structures.
The result of the docking calculations is a list of chemicals ordered by their predicted affinity for rER. All of the experimental rER binding chemicals appear in the first 27% of the list but are not ordered by their binding affinity. The choice of demarcation between predicted binding and non-binding chemicals is determined by the balance between false positives and false negatives and will be discussed. These results suggest that this approach has value as a prescreen for setting testing priorities.

The Conundrum !!!!!

- The application of modern experimental techniques to the study of chemical toxicity has let to an explosion of data that is relevant to the risk assessment process.
- Often that data is not quite the data one would want for evaluating risk.
- How does one use the existing data to obtain the information needed and/or to determine which is the key missing data?

Extrapolations for Evaluating the Risk Posed by Chemicals

- High Dose to Low Dose
- Route of Exposure to Route of Exposure
- Chemical to Chemical
- Species to Species
- Population Characteristics
  - Sensitive subpopulations
  - Life Stages
- Complex Exposure
  - Dose regime
  - Mixtures of Chemicals

The Target-Toxicant Paradigm

- The differential step in many mechanisms of toxicity may be generalized as the interaction between a small molecule (a toxicant) and one or more macromolecular targets.
- Targets include genetic material, receptors, transport molecules, enzymes and others.
- The difference in activity observed between chemicals acting through the same biological mode of action may then be understood as differences between their interactions with putative macromolecular targets.

Structure Based Screening of Protein Targets

Protein

- Initial protein structures obtained from www.pdb.org
- Relevant targets are optimized and/or modeled in MOE(CCG) using AMBER 94 Force-Field.

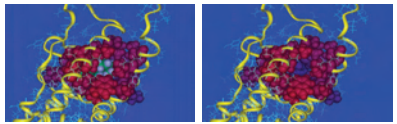
Ligands

- 3D optimized structures.

Estimate the Interactions - Docking

(1) eHiTS (Simbiosys), (2) FRED (Openeye) used in this study

Creating a Computational Protein Target



Ligand Bound — Ligand Removed Computationally

Comparison to Data for Binding to the Estrogen Receptor*

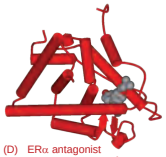
- 281 environmental chemicals were tested and 15 were found positive.
- All positives were weak

*from S Laws Laboratory

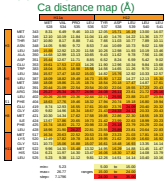
Results

(A) 3ERD – ER agonist

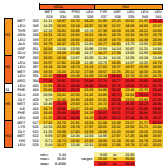
(B) 3ERT – ER antagonist



(C) ER agonist
Ca distance map (Å)



(D) ERα antagonist
Ca distance map (Å)



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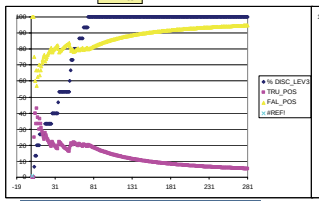

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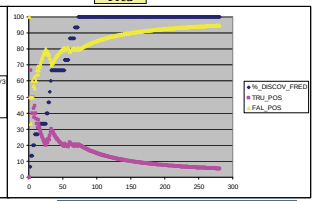
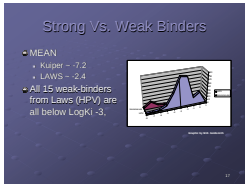


		EXPERIMENT	
5 Positives found		POS	NEG
PREDICT	POS	5	14
	NEG	10	252
10 Positives found		POS	NEG
	POS	10	41
	NEG	5	225
All positives found		POS	NEG
	POS	15	59
	NEG	0	207

		EXPERIMENT	
5 Positives found		POS	NEG
PREDICT	POS	5	18
	NEG	10	256
10 Positives found		POS	NEG
	POS	10	34
	NEG	5	222
All positives found		POS	NEG
	POS	15	58
	NEG	0	208

Receptor Structure

- Different Modes of Binding
  - Agonist Antagonist
- Different Proteins in the Same Prep
  - Alpha Beta
- We dock each chemical in both the agonist and antagonist mode of the receptor and into crystal structures of both the Alpha and Beta forms.



Discussion

The two methods used identify all the of the molecules that bind to an estrogen receptor within the first 73 in one case and 74 in the other case of a 281 chemical data base. There is considerable overlap of the false positives between the two methods but also some false positives that are unique to each method.

- In this case these methods work reasonably well for priority setting.
  - Because a number of the false negatives are the same in each method, there may be some systematic source of false negatives – a structural determinant.
  - Because there are a number of false negatives that are unique to each method then using the methods together will give a smaller false positive rate.

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