



# Macromolecular Modeling to Predict and Understand Toxicity: The Polycyclic Aromatic Hydrocarbons as a Test System

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research & development

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## Science Question

How do computational molecular modeling methods enable the incorporation of structural and mechanistic information into the extrapolations necessary for determining the potential risks of chemicals in the environment?

- A. Determining bioassay priorities
- B. Molecular level detail of mechanisms of action

## Research Goals

1. Apply computational molecular modeling methods to prioritize chemicals for testing (in conjunction with other short term assays.)
  - ❖ Adapting existing approaches
  - ❖ Develop new approaches
2. Supply insight into molecular level processes for toxicity that are not easily available experimentally.

## Approach

### The Target-Toxicant Approach

The interference of a xenobiotic or a metabolite in many of the relevant processes may be generalized as the interaction of a "small" molecule with a macromolecular target. The methods and the information available for modeling processes of this type are improving rapidly. On of the engines for this improvement is the pharmaceutical industry and the commercial need to develop new drugs. However, there are important differences between these two applications.

- ❖ To be a viable drug a molecule must be strong actor while environmental agents are often weak actors.
- ❖ If a drug company finds one or a few prospective agents, that is a success, we need to find all or almost all of the potential agents.
- ❖ False negatives are much more important than false positives in risk evaluation because the expectation is that positive chemicals will be tested.

		Measured	
		+	-
Predicted	+	2	8
	-	3	87

89% correct

An enrichment factor of 4

A false negative-true positives ratio of 1.5

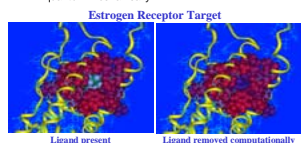
While the initial example seems better at first glance and may in fact be more advantageous for pharmaceutical discovery, the latter example is more useful for identifying potential chemical risks

		Measured	
		+	-
Predicted	+	5	45
	-	0	50

55% correct

An enrichment factor of 2

A false negative-true positives ratio of 0



### Molecular Modeling Methods for Studying Toxicity

#### Classical Methods

- ❖ Molecules are composed of atoms (spheres) connected by springs
- ❖ The interactions are classical, chemicals do not react
- ❖ Docking methods use this level of interaction

#### Quantum Mechanical Methods

- ❖ Molecules are described as a combination of nuclei and electrons
- ❖ The electronic distribution of the molecule or complex of molecules is determined
- ❖ Chemical reactions and other interactions that influence electronic distribution can be modeled

#### Mixed Models

- ❖ Part of the molecules is considered classically
- ❖ The reactive part of the molecular system is described quantum mechanically

**What is Docking?**  
The "best fit" for each chemical agent is calculated in the binding region of the macromolecular target. The best fit docking poses include both shape fitting and physical-chemical interactions.

**Rigid Docking**

- Fast computational screening tool, but accuracy is not ideal.
- Structure of the chemical agent has torsional flexibility.
- Geometrical structure of the active site of target is held fixed during the docking calculations.

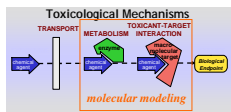
**Flexible Docking**

- Computationally intensive, but accuracy is more reliable.
- Structure of the chemical agent has torsional flexibility.
- Geometrical structure of the active site of target is flexible during docking calculations; the region can properly contour to fit chemical agents.

Examples of docking results: Good fit, Mediocre fit, No fit.

## Background

There is a conundrum in evaluating the risks posed by chemicals. Although there is an explosion in relevant data, often that data is not exactly what is needed. Situations are frequently encountered where the potential health and environmental effects of chemicals must be evaluated when all of the relevant information is not available. One rational approach for assessment under these circumstances is to estimate the relevant missing information from available information. Models of the biomolecular processes that constitute the potential mechanisms of action provide templates for this extrapolation.



## Why now?

**Biological Knowledge Advances:**

- Genetically modified organisms
- Transgenic animals
- Transgenic plants
- Environmental applications

**Computational Advances:**

- High speed computers
- Improved algorithms
- Improved applications

↓  
Interplay of Disciplines

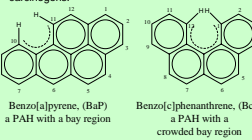
- Molecular level models for PAHs and other xenobiotics
- Ability to model biological processes and gene regulation
- Ability to apply chemical structure descriptors more easily to environmental mixtures

- The application of modern experimental techniques to the study of chemical toxicity has led 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?

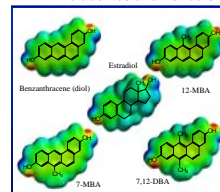
## Results

### What are Polycyclic Aromatic Hydrocarbons?

Polycyclic aromatic hydrocarbons (PAHs) are a pervasive class of anthropogenic chemicals. Many molecules in this class are found in the environment, primarily in mixtures. The testing of individual chemicals has shown great variation in carcinogenic activity for chemicals in this class. However many chemicals remain untested. Features of molecular structure that relate to the carcinogenic potency of these chemicals have been identified and a mechanism for carcinogenicity described. According to this mechanism the bay region diol-epoxide (DE) is the ultimate carcinogen. Consideration of this mechanism suggests the importance of three-dimensional molecular structure in addition to the electronic to electronic. In earlier studies we showed that PAHs with a crowded bay region formed a particularly potent subclass of carcinogens.



### Electrostatic Potential of PAH Metabolites and Estradiol



Observation of the structure and interaction potentials of PAHs indicate that some PAH metabolites are similar to estrogen.

Rigid docking studies using Glide4.0 with several crystal structures of the alpha estrogen receptor (ER $\alpha$ ) and the native ligands (bottom) and PAH metabolites.

	1L21 (etc)	1GWR (est)	3ERD (des)	3ERT (oh)	
					increase in docking affinity →
					<p><b>The dramatic differences obtained for the various ER crystal structures (left), especially for the ER antagonist tamoxifen, highlights the importance of flexible docking.</b></p> <p>ETC</p>

The figure indicates that the PAHs bind best to 1L21, the ER structure that was crystallized with tetrahydrochryseno diol (ETC) bound. ETC (structure above) is an ER agonist that has a chemical structure similar to PAHs and steroid hormones.

## Impact and Outcomes

This research supports the Agency's goals in the multi-year plans for Human Health and Endocrine Disruption. It addresses the significant Agency need for predictive models for hazard identification in the sub-areas of (1) QSAR and other computational approaches, and (2) high throughput screening.

This project supports two of the objectives of the NCCT as stated in the Computational Toxicology Framework: (2) develop predictive models for categorizing and prioritizing chemicals in the environment, and (3) improve quantitative risk assessments. The target-toxicant approach and molecular modeling in general can be used in conjunction with available experimental data to develop methods for screening environmental chemicals for toxicological effects on human health and ecosystems. These virtual screening tools will lead to more efficient chemical testing schemes and help streamline the risk assessment process. Active collaboration, including continuous feedback with experimental scientist at NHEERL and elsewhere enable this research.

The understanding of the processes that lead to chemical toxicity on the molecular level and the capability to perform virtual experiments on the computer will aid in the determination of cumulative risk due to multiple chemicals and other stressors.

## Conclusions

- ❖ Some metabolites of polycyclic aromatic hydrocarbons are likely to bind to the estrogen receptor
- ❖ Docking results using the crystal structures of the estrogen receptor with different ligands bound as targets are not the same
- ❖ Docking into the static binding site of a nuclear receptor may give unreasonable results (for weak binders) because the receptor are flexible.

## Future Directions

- ❖ Develop the Target-Toxicant paradigm for risk assessment
  - ❖ Methodologically
  - ❖ More diverse targets
- ❖ Use insight provided by this approach to consider cumulative risk
- ❖ See other posters

## References

- ❖ Glide version 4.0, Schrödinger, LLC, New York, NY, 2005.
- ❖ Rabinowitz, J.R., Little, S.B. and Brown, K.W., "Why Does 5-Methyl Chryseno Interact with DNA Like Both a Planar and a Non-Planar Polycyclic Aromatic Hydrocarbon? Quantum Mechanical Studies", International Journal of Quantum Chemistry, 88: 99-106, 2002.
- ❖ Rabinowitz, J.R., Little, S. B., Brown, K.W., Benzo[a]pyrene and benzo[c]phenanthrene: the effect of structure on the binding of water molecules to the diol epoxides. Chemical Research in Toxicology, 15: 1068-1076, 2002
- ❖ Rabinowitz, J.R., Little, S.B., Parsainelli, M. Research on the Application of Molecular Modeling Methods for the Prioritization of Assay Requirements and Classification of Chemicals in the Environment, presented at the ECVAM Workshop on Molecular Modeling, Ispra, Italy, February 2006.

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Long Term Goal II

