A Novel Molecular Modeling Framework to Predict Protein-Ligand Interactions to Detect Endocrine Disrupting Potential of Chemicals

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INTRODUCTION

There is a need for new approach methodologies (NAMs) to identify substances with an endocrine disrupting (ED) modality. Our goal is to provide an accurate, high-throughput, expandable and customizable tool for molecular modeling that can be used to detect potential ED modality. The interaction of chemicals with proteins is a key to investigate the disruption of endocrine pathways. Among the computational methods that could investigate such interactions, molecular docking is a technique that has a reasonable tradeoff between computational investment and accuracy of predictions. In the scientific process allowing for such a prediction to deal with the level of statistical complexity presented by the problem, molecular docking is used at midterm as a kernel around which a sophisticated framework must be built. Arguably, there is currently no publicly available "gold standard" computational framework entirely fit for purpose to handle this model; hence, our team is building our own computational framework.

METHODS

Fundamental parts of the framework consist of third-party databases[2] and software[3,4], while the linking of software and some analysis strategies were developed entirely by our team.

Gather experimentally derived 3D models of protein-ligand complexes involving proteins of interest and native or synthetic ligands

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Protein 3D models curation and clustering of similar protein conformations

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Prepare 3D protein models for molecular docking

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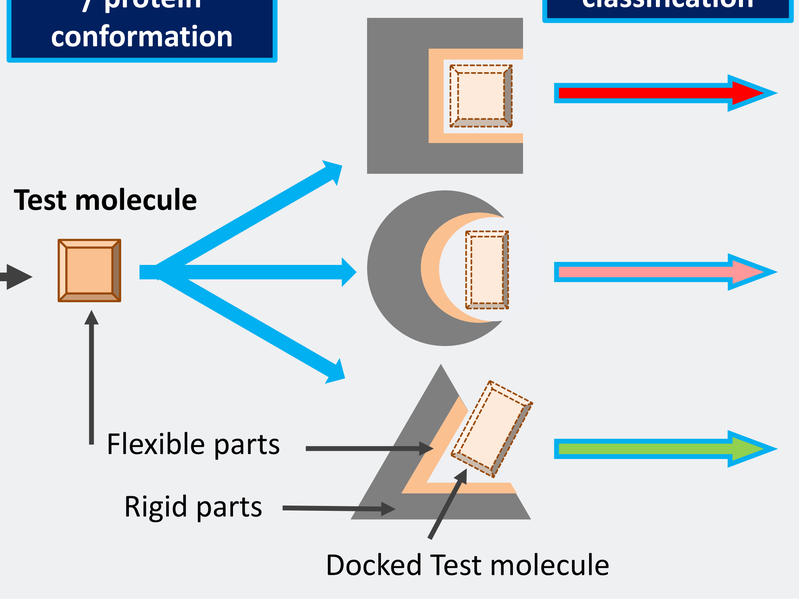
Ligand 3D models for molecular docking are prepared *de novo*

EATS biological target implemented in the workflow	Conformational clusters
Thyroid hormone receptor α (TRα)	2
Thyroid hormone receptor β (TRβ)	5
Estrogen receptor α (ERα)	18
Estrogen receptor β (ERβ)	10
Progesterone receptor (PR)	11
Androgen receptor (AR)	15

Flexible docking: *N* replicas / protein conformation

Set of protein conformations

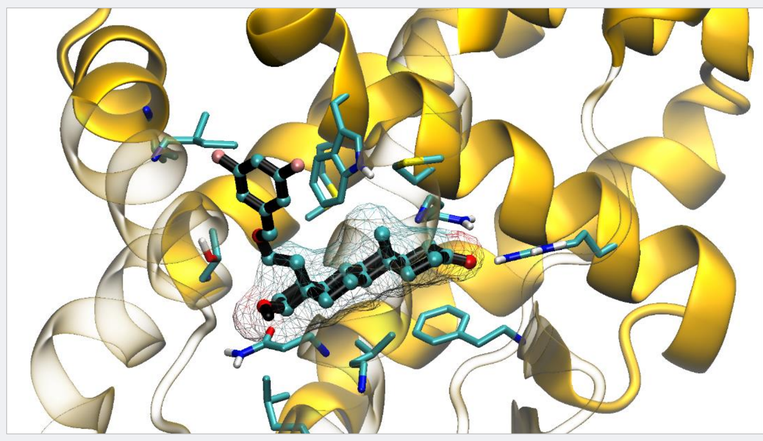
Analysis: Binding Profiles and classification



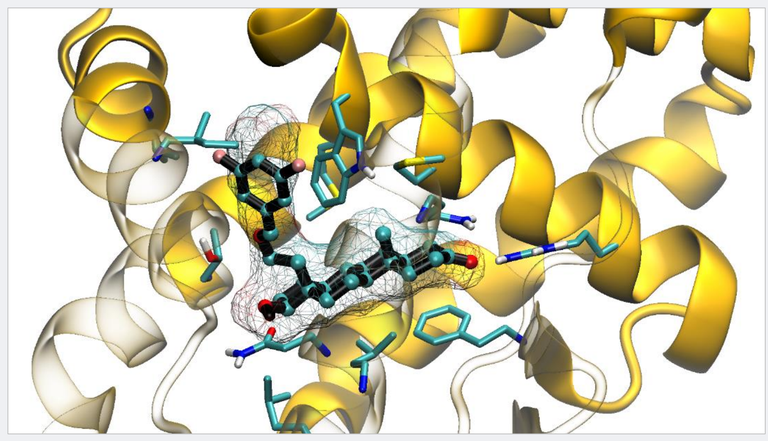
RESULTS OF EXTERNAL VALIDATION

Reliable predictions of protein-ligand binding and accurate quantification of interactions

Example of docking experiment results with compound ENM5744[5] (black sticks) in binding site BF-1 of target AR:



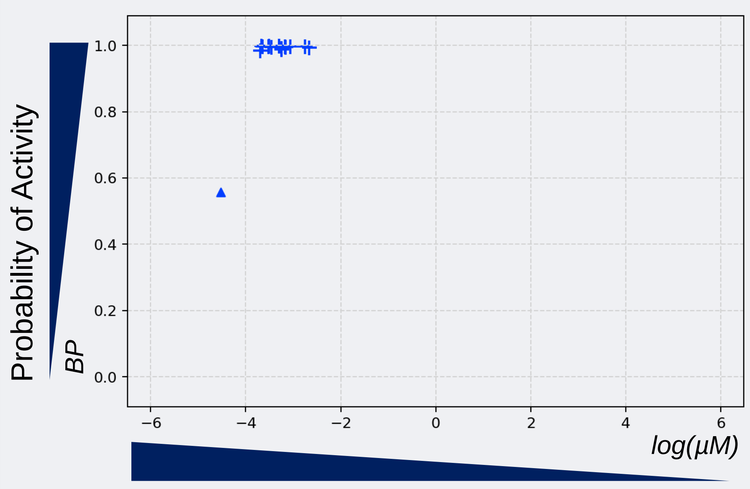
Comparison with experimental binding mode of Testosterone (wireframe molecular surface):
experimental $RBA_{Tes./ENM5477}$ = ~8750%
predicted $RBA_{Tes,/ENM5477}$ = 2677%



Comparison with experimental binding mode of compound ENM5477[5] (wireframe molecular surface):
experimental binding affinity ~0.0383 nM
predicted binding affinity 0.0294 nM
$RBA_{Experimental/Predicted}$ = 130%.

Novel scoring function

The scoring function termed Binding Profile (**BP**) accounts for thermodynamic and conformational properties of the docking experiment results.



Affinity & Interactions with Binding Site

Example of scoring of docking poses at target AR of compound ENM5744[5] (^) and of the native hormone Testosterone (+)

Robust statistical classification

Comparison of both Binding Profiles yielded a *p-value = 0.51* which is not significative at alpha-level 5%, thus the null hypothesis that compound ENM5477 is ligand to target AR cannot be rejected, i.e., the docking experiment is validated since the workflow recovered the experimental binding mode involving target AR and compound ENM5477.

The workflow has very high sensitivity

As a first example of external test-set data for validation, we used the available experimental binding modes across all protein targets with all ligands that are not native hormones – as binding modes of native hormones are used as training-set for the workflow.

Endpoint: protein binding	Total reference binding modes	Reference binding modes recovered at *1-alpha*		
		>=99.9%	99%	95%
TRα	3	3	3	3
TRβ	5	5	5	5
ERα	16	16	16	16
ERβ	19	19	19	19
PR	14	14	13	13
AR	32	32	31	30

The automatic workflow is also capable of:

Testing any organic compound

Investigating any protein target if experimental 3D models are available

Investigating different binding sites on the same target protein to provide fine mechanistic details about the molecular initiating event

Facilitating technical collaboration thanks to the open architecture of the workflow

CONCLUSION & PERSPECTIVES

We present a novel automatic workflow employing cutting-edge drug design modeling techniques, its high throughput makes it suitable to investigate the binding of chemicals to protein targets as the key initiating event in the scope of toxicological studies. Despite the high computational cost, our framework can provide its prediction results in matter of hours while covering numerous biological targets. Moreover, soon we will proceed to expand the applicability domain of biological targets investigated within the EATS classes and beyond, taking advantage of the already available experimental 3D data of protein conformations from public consortiums.
As demonstrated by the validation study using experimental binding modes, we are very confident about the high sensitivity of our methodology. We are currently working on assembling a reliable negative testing set in order to assess the specificity of the framework. We also intend to expand the investigated proteins' conformational space by simulation of molecular dynamics, thus allowing our framework to better evaluate the possibility of protein-ligand interactions in the scope of arbitrary *in-silico* toxicological studies.

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