

Identification of Estrogen receptor modulators using virtual screening (Drug discovery acceleration & Lead Optimization)

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Introduction

R&D production of new drugs has remained constant over the last number of years with major pharmaceuticals each launching roughly one new drug per year(1) Traditionally these molecules are found either by: Serendipitous Random Screening, as Natural products as Leads in Drug Design, Combinatorial Approaches, Rapid Throughput Screening, Structure-Based Drug Design – 'in silico' screening (Virtual screening). The goal is to accelerate the overall drug discovery process, or to "fail early" compounds that would fail further down the road in clinical or pre-clinical testing.

In this respect, we have validated and developed a Virtual High Throughput Screening protocol involving a suite of conformational and docking computational algorithms optimized to carry out screening of candidate compounds for estrogen receptor antagonist activity, using X-ray crystal models of the Estrogen Receptor alpha (ER α).

Significance of ER:

- Estrogens regulate cell growth, differentiation & development of reproductive tissues in men and women.
- Maintain bone density preventing osteoporosis.
- Exerts anti-atherosclerotic effects which lowers Cholesterol levels.
- Involved in many CNS effects (Parkinson's) and implicated in Alzheimers
- 60% of primary breast cancers contain ER- alpha.
- Estrogens are mitogenic for ER-positive breast cancer cells.

Modulation of the Estrogen Receptor: Antiestrogens

Antiestrogens compete with endogenous ligands for ER binding and render the ER inactive.

- Antiestrogenic side chain of SERM (Selective Estrogen Receptor Modulator) interacts with ASP351 and rearrangement of Helix 12 blocks co-activator attachment.
- Pure antagonists also down-regulate ER.
- Antiestrogens up-regulate E-Cadherin which is involved in cell adhesion. Loss of cell adhesion (E-Cadherin) causes tumour metastasis(2).

Figure 1: Antiestrogens vs Estrogens



Methods: 'In House' Virtual Screening Protocol

To evaluate the strengths and limitations of our vHTS and to compare several widely used methods (FlexX, FRED, eHits), we firstly assessed the ability of our computational drug design suite in retrieving a set of potential modulators that would exhibit selectivity towards the estrogen receptor alpha (ER α). The enrichment and hit rate achieved by our protocol was compared to these commercially available programs.

Secondly we assessed the ability of our virtual screening suite in identifying novel estrogen receptor alpha modulators from a compound library of 40000 compounds.

Validation 1

- A haystack built from 1000 'drug-like' compounds from Maybridge HTS collection (Maybridge plc HITS Kits) was seeded with a set of known actives (35 Estrogen Receptor Modulators taken from literature).
- Dataset was converted from 2D-3D using CORINA with 10 conformers generated for each compound.
- Targets 3ERT, 1ERR, 1UOM prepared for docking from PDB. Docked and scored with 'in house' programs LIGIN and LPC(3). Re-scored using 10 scoring functions.
- Compared with commercial software FRED (Openeye Software), FlexX (Tripos Inc.)

Validation 2

- A haystack built from 1000 'drug-like' compounds from Maybridge HTS collection (Maybridge plc HITS Kits) was seeded with a set of known actives (40 Estrogen Receptor Modulators taken from literature).
- Dataset was converted from 2D-3D using CORINA with 10 conformers generated for each compound.
- Target 3ERT prepared for docking from PDB. Docked and scored with 'in house' programs LIGIN and LPC. Re-scored using Chemscore scoring function.
- Compared commercial software FRED (Openeye Software), eHits (Symbiosis Inc.)

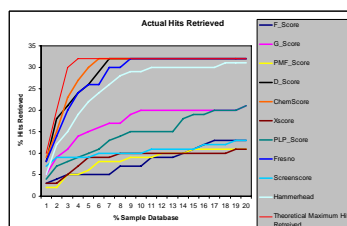
Screen 1:

- ASDI Database of 40000 compounds downloaded.
- Dataset was converted from 2D-3D using CORINA with 10 conformers generated for each compound.
- Targets 3ERT, 1ERR, 1UOM prepared for docking from PDB. Docked and scored with 'in house' programs LIGIN and LPC. Re-scored using Chemscore scoring function.
- Hits clustered using MOE cluster fingerprints application.

Results

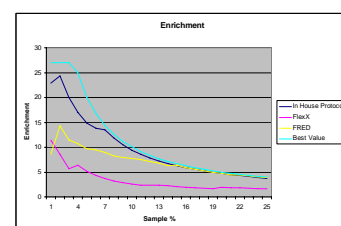
Validation 1 Results:

Hits retrieved using several known scoring functions to re-score the database of ligands



Applying Chemscore scoring function retrieves most actives.

Enrichment rate using several protocols. Enrichment = Hit Rate in Subset / Hit Rate in Database



Enrichment rate is highest using 'in house' protocol.

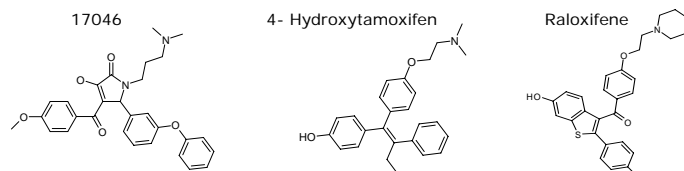
Validation 2 Results:

Subset Size %	1	2	3	4	5
eHits	12.5	11.25	9.16	8.75	7
FRED	7.5	11.25	12.5	11.88	9.5
In House' Protocol	20	17.5	18.33	17.5	15
Best Value	25	25	25	25	20

Enrichment rates of 'in house' protocol outperform others

Screen 1 Results:

- A set of 60 compounds were ordered from ASDI priority powders database and will be tested for activity using MTT assay.
- Example of compound retrieved as hit compared with two known antagonists:



The execution time to run the protocol outlined in validation 1 was 30 minutes using a 160 node linux cluster.

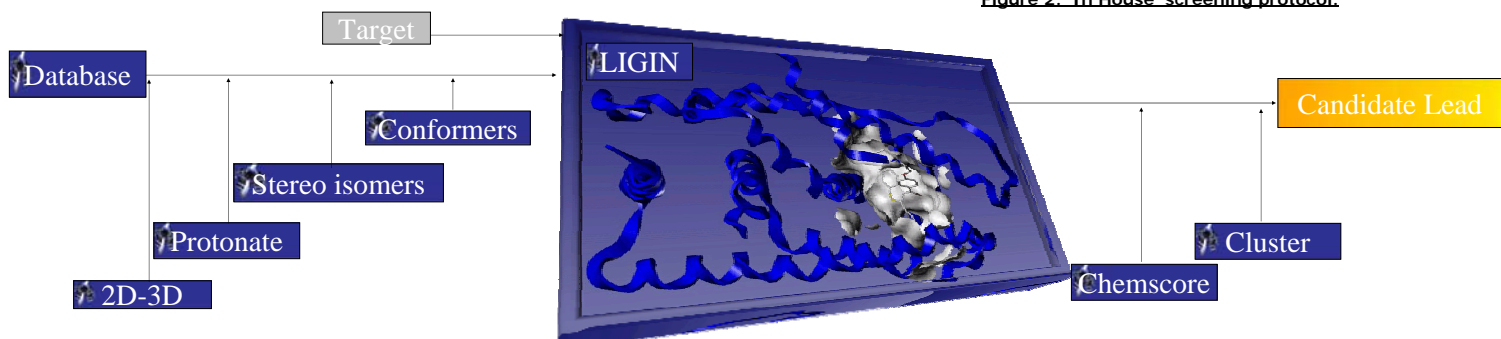
Current work:

The addition of calculation of protonation states and generation of stereo isomers for each compound has been implemented and its effect on enhancing the enrichment rate is currently being validated. An overview of the present screening process is outlined below in Figure 2.

References:

1. Smith, A. *Nature* **2002**, *418*, 353-463.
2. Steffi Oesterreich et al. *Cancer Research* **2003**, *63*, 5203-5208.
3. Sobolev V., Wade R.C., Vriend G. and Edelman M. *Proteins* **1996**, *25*, 120-129.

Figure 2: 'In House' screening protocol:



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