Pharmacophore-based in silico screening for bio-affinity profiling of ligands

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Success Rate Is Too Low

Development phases of a new drug

- Preclinical studies
- Phase I clinical study
- Phase II clinical study
- Phase III clinical study
- Market launch

> 100,000 new compounds

Years

Success Rate

800 Mio $
Efficiency Deficit ...

Prediction of failure in early stage

>> 100,000

1

800 Mio $

150 Mio $
Reasons For Attrition

Phase III failures 1992-2002 ...

- Cipemastat
- Lazabemide
- Lotrafiban
- Sorivudine
- Tasosartan

... n=26

- 53% clinical efficacy
- 4% pharmacokinetics
- 4% diverse
- 4% portfolio
- 35% side effects & toxicity

One Answer: In Silico Screening

1D Filter
- properties
- fingerprints
  e.g. MW 200-500
  Ro5 / Lipinski

2D Filter
- topology, mol. graphs
- (red. graphs, FTrees, ...)

3D Filter
- 3-point pharmacophores
- distance hashing

3D Fitting
- flexible
- pre-computed conformers

computationally expensive
Usual Virtual Screening Protocol

10^x molecules against one target results in a hit list
Why Not Do This?

10^x molecules against 10^x targets

... needs a large number of models!
What Is A Pharmacophore?

“A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response.”

Example One

Phenolphthalein, Crystal violet, Methylene blue

Adrenalin, Noradrenalin, Isoprenalin, Dopamin, Amphetamin

Pharmacophores?
Representation of Pharmacophores

Fragment-distance (topology)

Chemical features in the 3D space

Amide

Carboxylate

Imidazole

H-bond donor

H-bond acceptor

Aromatic ring
Feature-based Pharmacophore Models

Totality of universal chemical features that represent a defined binding mode of a ligand to a bio-molecular target

Features: Electrostatic interactions, H-bonding, aromatic interactions, hydrophobic regions, coordination to metal ions ...
Why Use Pharmacophore Models?

Universal
- Pharmacophore models represent chemical functions, valid not only for the currently bound, but also unknown molecules

Computationally Efficient
- Due to their simplicity, they are suitable for large scale virtual screening ($>10^9$ compounds, also in parallel settings)

Comprehensive & Editable
- Selectivity-tuning by adding or omitting chemical feature constraints, information can be traced back
How To Build Pharmacophore Models?

• Starting from ligand information
  – Exploration of conformational space
  – Multiple superpositioning experiments
  – DISCO, Catalyst, Phase, MOE, Galahad ...

• Starting from 3D target information
  – GRID interaction fields: Convert regions of high interaction energy into pharmacophore point locations & constraints
    [S. Alcaro et al., Bioinformatics 22, 1456-1463, 2006]
  – Start from target-ligand complex: Convert interaction pattern into pharmacophore point locations & constraints
Let’s have a look ...
**Implemented Procedure**

1. Detect ligand and clean-up the binding site in the protein (all amino acids within 7Å distance from the ligand)
2. Interpret hybridization status and bond types in the ligand
3. Perform chemical feature recognition for the ligand (H-bond donor, H-bond acceptor, positive ionizable, negative ionizable, hydrophobic, aromatic ring, metal ion coordination)
4. Search for corresponding chemical features of the protein
5. Add interaction features to the model only if a corresponding feature pair is found in the complex
6. Add excluded volume spheres for opposite hydrophobic features

LigandScout Graphical User Interface
Binding Mode Specificity

One pharmacophore model accounts for one binding mode ...

How to analyze and align these objects?
Alignment By Pharmacophore Points

Methotrexate

Dihydrofolate

Wrong

Correct

Böhm, Klebe, Kubinyi:
Wirkstoffdesign (1999) p. 320f
Alignment By Pharmacophore Points

1RX2

1RB3
Pharmacophoric Alignment

molecule pharmacophore → best pairing → 3D rotation (Kabsch) → Superposition

Is pairing valid?
If not, remove invalid pairs and retry

How To Find The Best Pairs ...

Hungarian Matcher (Marrying Problem)


Hungarian Matching

How to define the pharmacophore feature matching cost (similarity)?

- Use only few feature types
- Create selectivity by defining geometric relations

=> Solution: Encode geometry in each feature!
Typed Distance Shells

- **Acceptor**: 0 | 0 | 1
- **Donor**: 0 | 1 | 1
- **Lipophilic**: 0 | 1 | 1
Distance Characteristics

Result: Best matching pairs for each feature

Final step: 3D rotation using Kabsch algorithm
Flexible Alignment

- Generation of conformer ensemble (OMEGA 2.0)
- Alignment experiment on bio-active conformation

Example: Understand Common Features ...

Example: RET Kinase Inhibitors

2ivv

2ivu
Generation of Shared Feature Pharmacophore

RET-Kinase inhibitor
ZD62015, bound conformation
(pdb entry 2ivu)
Generation of Shared Feature Pharmacophore

RET-Kinase inhibitor PP12014, bound conformation, pdb entry 2ivv
Shared Feature Pharmacophore
Merged Feature Pharmacophore
Pharmacophore-based Alignment
Ligand Profiling Case Study: Antivirals

- 5 viral targets
- 50 pharmacophore models
- 100 antiviral compounds

Will their activity profiles be predicted correctly?
# Ligand Profiling: Targets

<table>
<thead>
<tr>
<th>Target</th>
<th>Disease</th>
<th>Function</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV protease</td>
<td>HIV infection, AIDS</td>
<td>Cleavage of gag and gag-pol precursor polyproteins into functional viral proteins</td>
<td>Inhibition at active site</td>
</tr>
<tr>
<td>HIV reverse transcriptase (RT)</td>
<td>HIV infection, AIDS</td>
<td>Synthesis of a virion DNA, integration into host DNA and transcription</td>
<td>Inhibition at allosteric site</td>
</tr>
<tr>
<td>Influenza virus neuraminidase (NA)</td>
<td>Influenza</td>
<td>Viral envelope glycoprotein, cleave sialic acid residues for viral release</td>
<td>Inhibition at active site</td>
</tr>
<tr>
<td>Human rhinovirus (HRV) coat protein</td>
<td>Common cold</td>
<td>Attachment to host cell receptor, viral entry, and uncoating</td>
<td>Binding in hydrophobic pocket (capsid stabilization)</td>
</tr>
<tr>
<td>Hepatitis C virus (HCV) RNA polymerase</td>
<td>Hepatitis C</td>
<td>Viral replication, transcription of genomic RNA</td>
<td>Inhibition at various allosteric sites</td>
</tr>
</tbody>
</table>
## Results Matrix

**Ligand-directed Analysis**

<table>
<thead>
<tr>
<th>Ratio ≥ 1</th>
<th>90% of the compounds correctly predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio &lt; 1</td>
<td>8% more often predicted for one specific false target than for correct one</td>
</tr>
</tbody>
</table>

For 2% of the compounds no activity prediction possible
## Pharmacophore-directed Analysis

<table>
<thead>
<tr>
<th>HIV protease</th>
<th>HIV RT</th>
<th>Influenza NA</th>
<th>HRV coat protein</th>
<th>HCV polymerase 1 2 3</th>
</tr>
</thead>
</table>

- **Model with highest selectivity:**
  - 100% of actives (HCV polymerase 1), no other compounds
  - 100% active and 0% inactive compounds in hit list

- **Model with 85% hit rate:**

- **Model with lowest selectivity:**
  - 70% of actives (HIV RT), but 75% from one specific false target (HRV coat protein)
  - 40% active and 60% inactive compounds in hit list

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Introduction in Chemoinformatics, Strasbourg, June 3, 2009
Underlying Screening Platform

PipelinePilot Script & Catalyst™ DB Search

K. Chuang
J. Benedict
N. Triballeau-Hugounencq
Rémy D. Hoffmann
Web Based Parallel Screening Platform
Web Based Parallel Screening Platform
How Can This Information Be Used?

• Pharmacophores only give geometric fit values

• Don’t forget about other parameters:
  – solvation / entropy
  – kinetic parameters
  – conformational strain energy …

• Pharmacophores are excellent filter tools for rapid pre-screening of very large compound DBs
First published examples of applications of extensive parallel screening approach based on pharmacophores

- Multitude of pharmacophore models (up to several thousand ...)
- Large set of molecules (up to several million ...)

Results indicate

- Correct assignment of selectivity in most cases
- Independent of search algorithms used

Fast, scalable *in silico* activity profiling is now possible!
The Inte:Ligand Pharmacophore Database

~ 300 unique targets ready to use*

- Represented in
  ~ 200 ligand-based pharmacophore models
  ~ 2200 structure-based pharmacophore models

- Covering a selection of all major therapeutic classes
- Contains anti-target models for finding adverse effects
- Categorized according to the pharmacological target

* out of ~650 categorized by May 2009
From a practical point of view ...

How to avoid toxicity and unfavorable ADME/Tox profiles in your own med chem projects?
SOSA: New Leads from Old Drugs

SOSA = Selective Optimization of Side Activities

1 – Start the screening with a limited set of carefully chosen, structurally diverse, drug molecules (a smart library of about 1000 compounds). As for these drugs bioavailability and toxicity studies have already been performed and as they have proven usefulness in human therapy, all hits that will be found are “drug-like”!

2 – Optimize hits (by means of traditional or parallel chemistry) in order to increase the affinity for the new target and decrease the affinity for the other targets. The objective is to prepare analogues of the hit molecule in order to transform the observed “side activity” into the main effect and to strongly reduce or abolish the initial pharmacological activity.
Activity profile inversion of minaprine

Selective Optimization of a Side Activity yields a new lead

Minaprine (Cantor®)
- Dopaminergic: +++
- Serotoninergic: ++
- Cholinergic: 1/2+

Modified Analogue
- Dopaminergic: o
- Serotoninergic: o
- Cholinergic: +++++

The SOSA Approach: A Historical Example

Affinity for muscarinic M1 receptors

minaprine
Ki = 17,000 nM

Ki = 550 nM

Ki = 50 nM

Ki = 3 nM

The rationale behind the SOSA approach lies in the fact that, in addition to their main activity, almost all drugs used in human therapy show one or several side effects.

In other words, if they are able to exert a strong interaction with the main target, they exert also less strong interactions with some other biological targets. Most of these targets are unrelated to the primary therapeutic activity of the compound.

The objective is then to proceed to a reversal of the affinities, the identified side effect is becoming the main effect and vice-versa.
**SOSA: Patentability – Risk of interference**

- The risk with the SOSA approach is to prepare a molecule already synthesized by the initial inventors and their early competitors.

- In fact, in optimizing another therapeutic profile than that of the initial one, the medicinal chemist will rapidly prepare analogues with chemical structures very different from that of the original hit.

- As an example, a medicinal chemist interested in phosphodiesterases and using diazepam as lead, will rapidly prepare compounds which are out of scope of the original patents, precisely because they exhibit dominantly PDE inhibiting properties and almost no more affinity for the benzodiazepine receptor.
**SOSA: Safety & Bioavailability**

- During years of practicing SOSA approaches, we observed that starting with a drug molecule as lead substance in performing analogue synthesis, increased notably the probability of obtaining safe new chemical entities.

- In addition most of them satisfy Lipinski’s\(^1\), Veber’s\(^2\), Bergström’s\(^3\), and Wenlock’s\(^4\) recommendations in terms of solubility, oral bioavailability, and drug-likeness.

Conclusions ...

- SOSA together with parallel pharmacophore-based virtual screening is a straightforward and rapid method for the generation of new lead compounds.
- Together with informatics-based molecular building tools, optimized design of novel and promising compounds will become feasible.
- Assessment of risks in later development stages becomes possible on a rational & transparent basis.
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