Pharmacophore Approaches In Drug Discovery

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Non-HTS Hit Recognition

A retrospective analysis of the drug discovery routes other than HTS highlights four efficacious strategies giving access to hits and/or lead compounds:

- **Analogue design** modification of existing active molecules to create an improved medicine (or new intellectual property)
- **Serendipitous observations** of unexpected clinical or pharmacological activities (trinitrine, hypoglycemic sulfonamides, sildenafil, etc.)
- **Rational design** of drugs resulting from the knowledge of the molecular mechanism and its role in disease (captopril, cimetidine)
- **Selective optimization of side activities** of known drugs on new pharmacological targets (SOSA Approach)

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COMMENTARY

New uses for old drugs

It takes too long and costs too much to bring new drugs to market. So let's beef up efforts to screen existing drugs for new uses, argue Curtis R. Chong and David J. Sullivan Jr.

Fast, affordable drug development is a vision that contrasts sharply with the current state of drug discovery — which also neglects too many diseases of the poor. An analysis[1] of 68 approved drugs estimated that it takes an average of 15 years and US$800 million to bring a single drug to market. And despite a doubling in research spending by the US National Institutes of Health (NIH) to $27 billion in 2003, the number of new drugs approved by the US Food and Drug Administration (FDA) each year remains constant at 20–30 compounds[2]. At this rate it will take more than 300 years for the number of drugs in the world to double.

The current costly and time-consuming paradigm of drug discovery is ill-equipped to combat rapidly emerging diseases, such as avian flu, drug-resistant pathogens and diseases that have a small financial market. One solution is to identify new uses for existing drugs. As the pharmacologist and Nobel laureate James Black said, "the most fruitful basis for the discovery of a new drug is to start with an old drug." Because existing drugs have known pharmacokinetics and safety profiles and are often approved by regulatory agencies for human use, any newly identified use can be rapidly evaluated in phase II clinical trials, which typically last two years and cost $13 million[3]. In this way, drug developers can bypass almost 40% of the overall cost of bringing a drug to market by eliminating much of the toxicological and pharmacokinetic assessments[3].

This back-to-basics approach is growing in popularity. At least 17 existing drugs are in various stages of clinical and animal testing for new uses (see Supplementary information), and a further 24 are already being remarketed by the pharmaceutical industry for new uses[3]. Although most successful crossovers have been the result of chance observations or educated guesses, exceptions include the antibiotic ceftriaxone, which is a potential treatment for amyotrophic lateral sclerosis[4], and whose new activity was discovered following the screening of 1,040 compounds from the National Institute of Neurological Disorders and Stroke (NINDS) custom collection in Gaylordsville, Connecticut. In the past, individual labs were limited to screening perhaps hundreds of compounds. Now, clinical drug collections like the NINDS library and the Prestwick Chemical Library in Washington DC offer more than 1,000 approved drugs for small-scale laboratory screening. In our view, what is needed is a more systematic approach to drug rediscovery that takes the most valuable resources to the next level.

Historically, 'repurposing' old drugs has proved successful in bringing new therapies to the developing world. Today, even with the billions of research dollars available to create new drugs through public–private partnerships, and the promise of genomic data, there remains an enormous unmet need for therapies for neglected diseases[5]. A recent example of a repurposed drug is miltefosine, initially developed for breast cancer but now used for treating visceral leishmaniasis[6]. This disease is caused by a sand-fly-transmitted parasite and kills an estimated 500,000 people each year. In fact, miltefosine failed phase II testing for tumour reduction and the drug was never approved by the FDA for cancer therapy. However, *in vitro* and animal studies indicated anticancer activity, and phase II trials confirmed miltefosine as a viable treatment for visceral leishmaniasis[6].

"The most fruitful basis for the discovery of a new drug is to start with an old drug."

Cost cutting

Cost is one reason to revisit existing drugs: roughly 1,000 of the 10,000 or so drugs ever tested in clinical medicine are covered by patents, so most drugs can affordably be redeployed in the developing world. Safety is another compelling reason. Phase IV clinical studies, which monitor post-marketing safety, cost around $100 million per drug to perform in developed countries[7] and are nearly impossible in countries without an established healthcare infrastructure. Because many existing drugs have undergone phase IV surveillance in millions of patients, the same stringent safety standards required by users in developed countries can be offered to patients with neglected diseases in the developing world.

Despite the promise of finding new uses for existing drugs, a comprehensive collection of the approximately 9,990 drugs known to clinical medicine does not exist. This number includes 2,933 unique drugs approved by the FDA since 1938 (*ref. 7*), 1,107 drugs in the 2006 FDA Orange Book, 888 drugs in the 2006 Physician Desk Reference, and 7,057 drugs that are either approved abroad or have entered phase II clinical trials, as indicated by a US Adopted Name or International Non-proprietary Name[8]. Excluding antiseptics, pharmaceutical aids, therapeutic plant or animal extracts, and vaccines, we estimate that there are 8,850

645

Sir James Black

Chong & Sullivan, *Nat. Drug Discov.* 2007, 448, 645-646

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SOSA: New Leads from Old Drugs

SOSA = Selective Optimization of Side Activities

1 - Start screening with a limited set of carefully chosen, structurally diverse, drug molecules (a smart library of about 1000 compounds). Already bioavailability and toxicity studies have 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.

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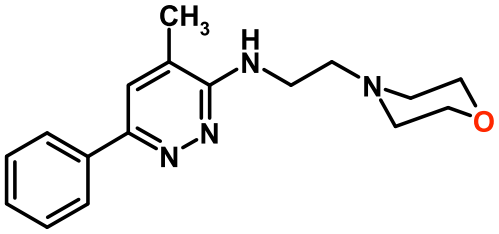
Activity Profile Inversion of Minaprine

Selective Optimization of a Side Activity yields a new lead

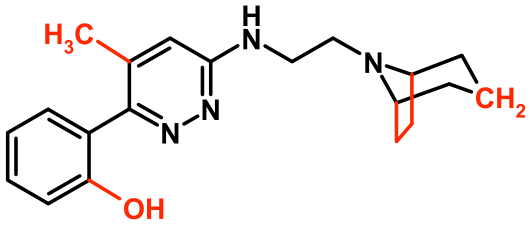


Minaprine (Cantor®)

Dopaminergic: +++

Serotoninergic: ++

Cholinergic: 1/2+



Modified Analogue

Dopaminergic: o

Serotoninergic: o

Cholinergic: ++++

Wermuth, C. G. Il Farmaco 1993, 48, 253-274.

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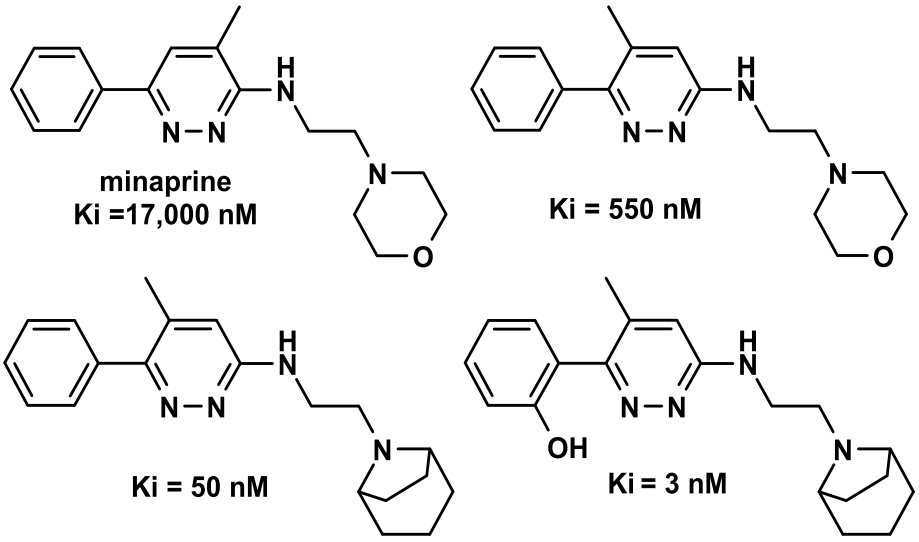
Activity Profile Inversion of Minaprine

Affinity for muscarinic M1 receptors



Wermuth, C. G. Il Farmaco 1993, 48, 253-274.

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Rationale of the «SOSA» Approach

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*.

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SOSA: Patentability & Interference Risk

• 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 phospho-diesterases 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.

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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.

1) Lipinski, C. A. et al. Adv. Drug. Delivery. Rev. 2001, 46, 3-26.
2) Veber, D. F.; et al. J. Med. Chem. 2002, 45, 2615-2623.
3) Bergström, C. A. et al. J. Med. Chem. 2003, 46, 558-570.
4) Wenlock, M. C. et al. J. Med. Chem. 2003, 46, 1250-1256.

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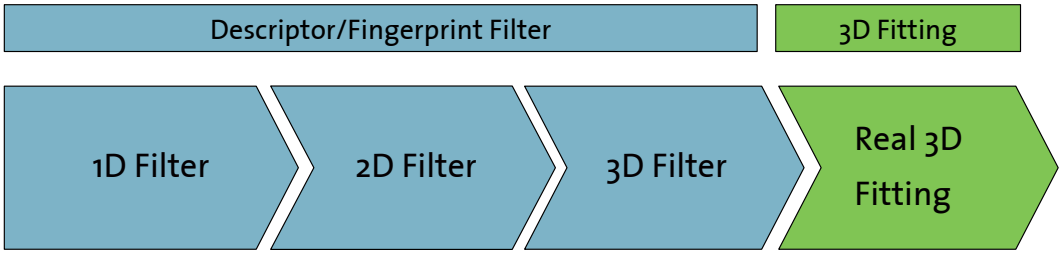
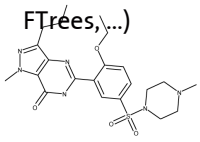
One Solution: In Silico Screening



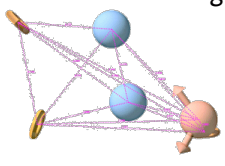
1D Filter
- properties
- fingerprints

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

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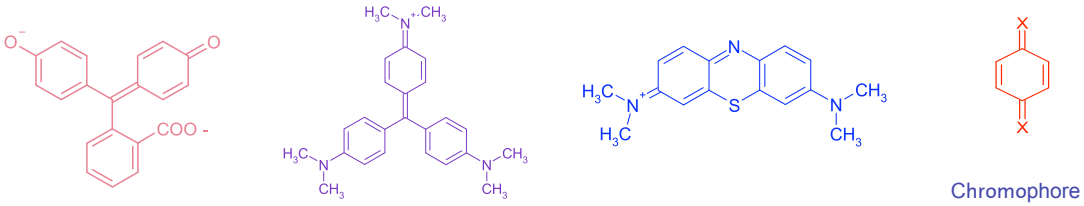
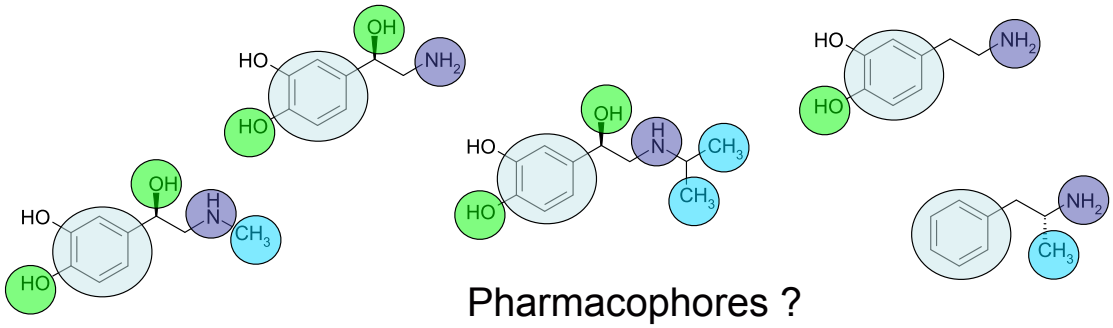
Example One

Phenolphthalein, Crystal violet, Methylene blue



Adrenalin, Noradrenalin, Isoprenalin, Dopamin, Amphetamin



Pharmacophores ?

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Our Aim: Predict Activity Pattern ...

- **Modeling of all relevant targets**
  - responsible for drug action and side effects
  - build feature-based pharmacophore models
- **Compile all models (+ relevant info) into a database**
  - Activity profiling of leads / drug candidates
  - Determination of side effects / bio-hazards
- **Use this system for development of novel interesting lead molecules and drug candidates**

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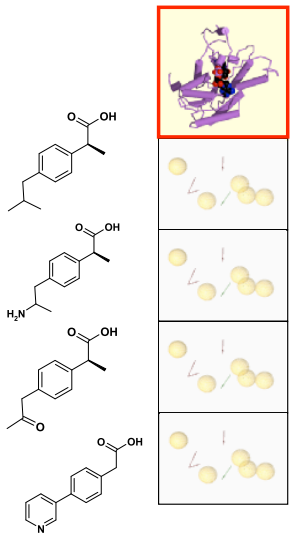
The Usual Virtual Screening Protocol



10^x molecules against one target

Hypothesis Data Spreadsheet: ACE-hypogen 20 Compounds Pag

Row	Name	Activ	Uncert	Color	Estimate	Error	MolWt	Principal	MaxOmitFeat
1	ala-gly	2.5e+06	3.0				146.146		
2	ala-his	9e+06	3.0				226.235		
3	ala-leu	1.6e+06	3.0				202.253		
4	ala-pro	270000	3.0				186.21		
5	ala-val	300000	3.0				188.226		
6	arg-ala-pro	16000	3.0				342.397		
7	glu-ala-pro	360000	3.0				315.326		
8	gly-asp	9.2e+06	3.0				190.155		
9	gly-glu	5.4e+06	3.0				204.182		
10	gly-lys	5.4e+06	3.0				203.241		
11	gly-phe	450000	3.0				222.243		
12	ile-pro	150000	3.0				228.291		
13	ile-tyr	3700	3.0				294.35		
14	leu-ala-pro	2300	3.0				299.369		
15	nleu-ala-pro	700	3.0				299.369		
16	phe-ala-pro	4200	3.0				333.387		
17	phe-pro-pro	78000	3.0				359.424		
18	pro-pro	7.5e+06	3.0				212.248		
19	val-pro	420000	3.0				214.264		
20	val-trp	1700	3.0				303.36		

results in a hit list

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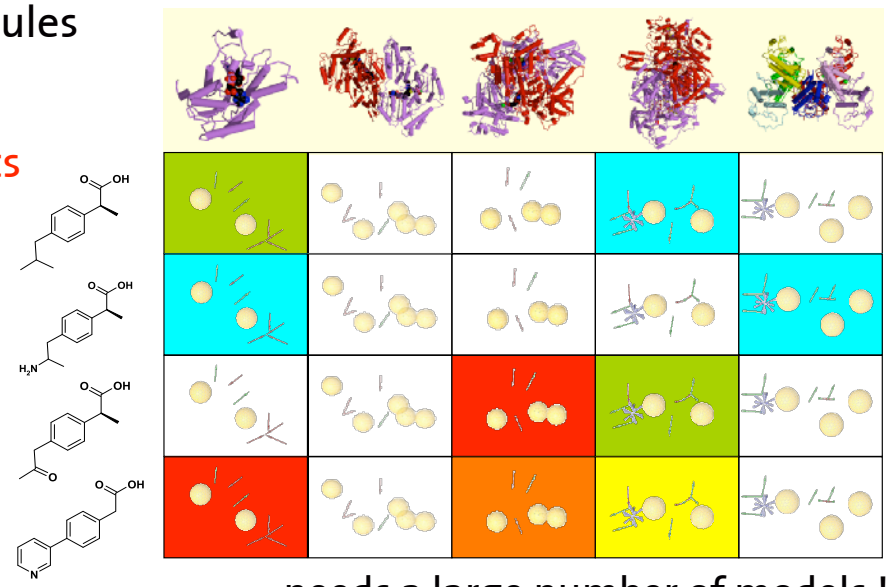
Why Not Do This ?

10^x molecules

against

10^x targets



... needs a large number of models !

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What Is A Pharmacophore ?

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

C.-G. Wermuth et al., *Pure Appl. Chem.* 1998, 70: 1129-1143

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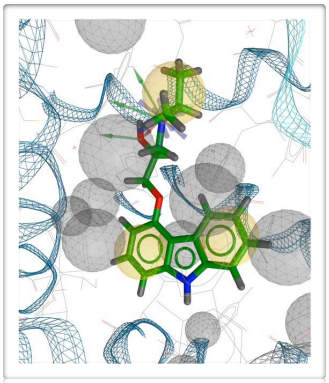
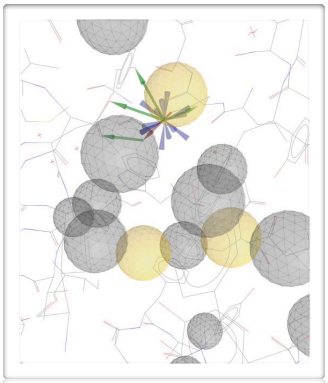
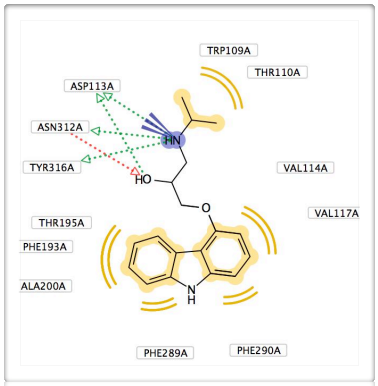
Feature-based Pharmacophore Models

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 ...







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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 easily traced back

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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
    [G. Wolber et al., J. Chem. Inf. Model. 45, 160-169, 2005]

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Let's have a look ...

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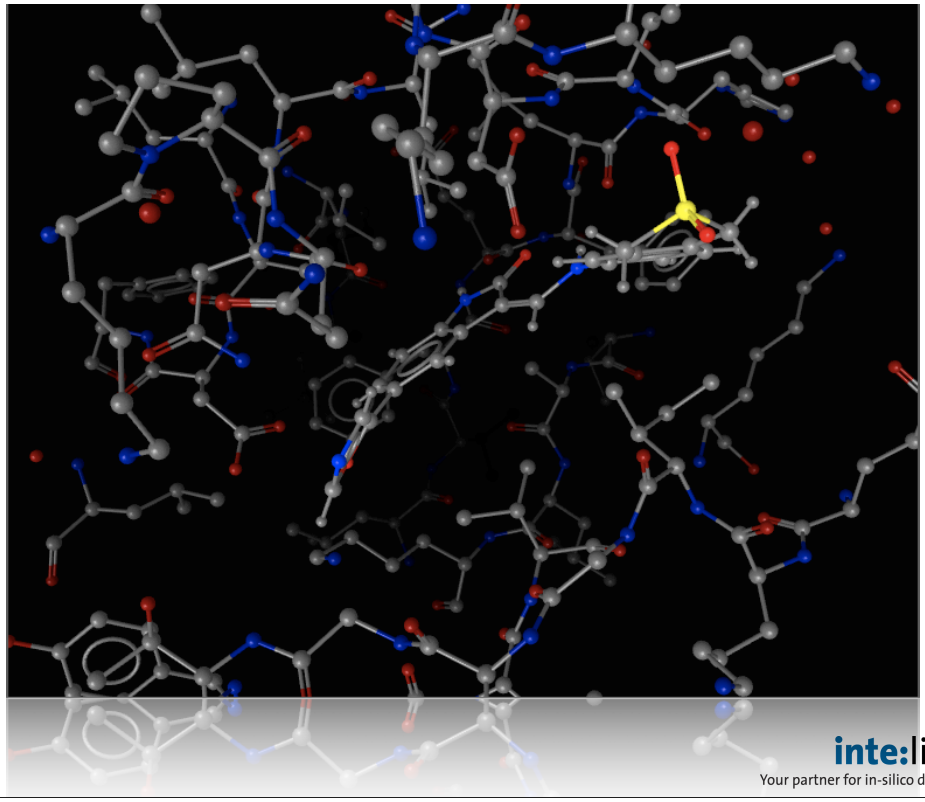

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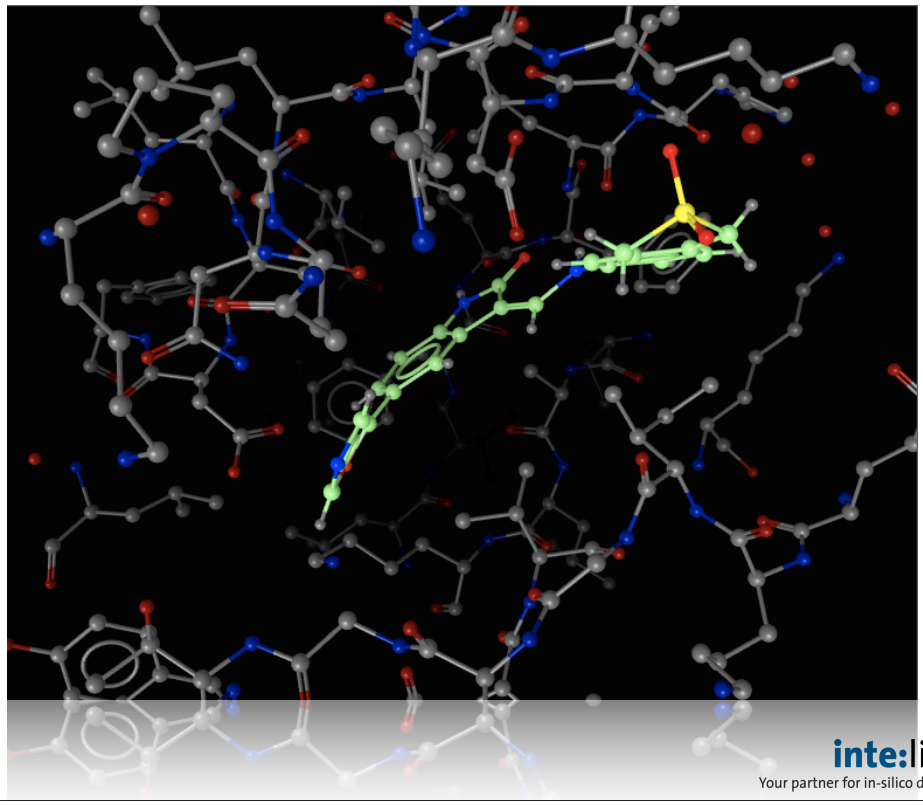

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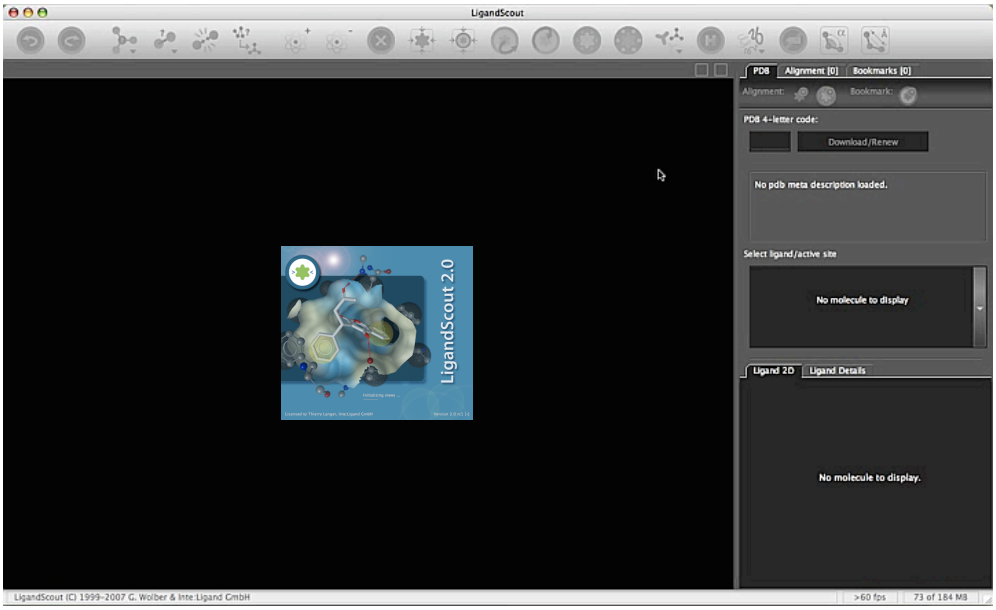
Implemented Procedure

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

G. Wolber, T. Langer: J. Chem. Inf. Model. 45 , 160-169 (2005)

LigandScout Graphical User Interface



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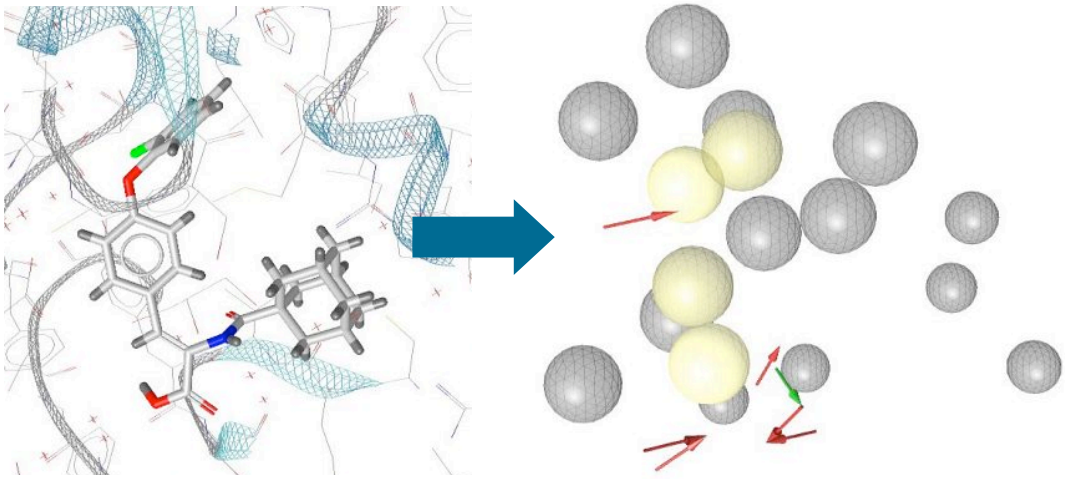
Binding Mode Specificity

One pharmacophore model accounts for one binding mode ...



How to analyze and align these objects ?

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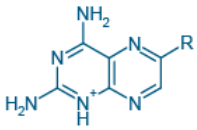
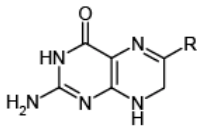
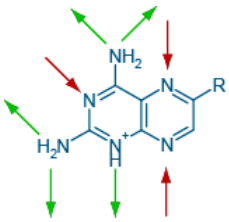
Alignment By Pharmacophore Points

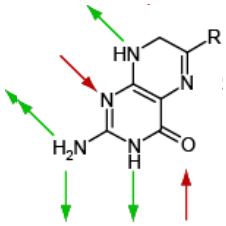


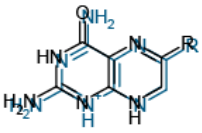
Methotrexate



Dihydrofolate

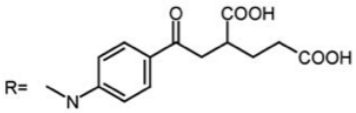






Wrong

Correct



Böhm, Klebe, Kubinyi:
Wirkstoffdesign (1999) p. 320f

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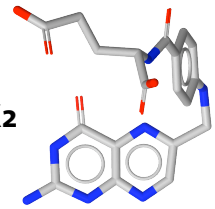
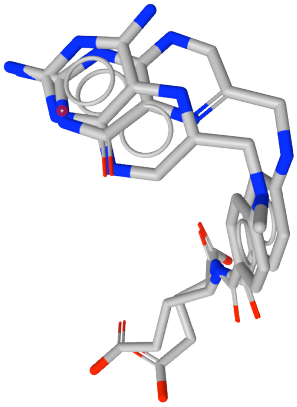
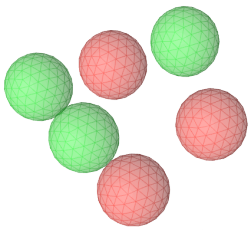
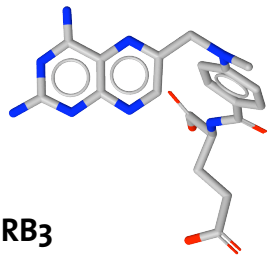
Alignment By Pharmacophore Points

1RX2









1RB3

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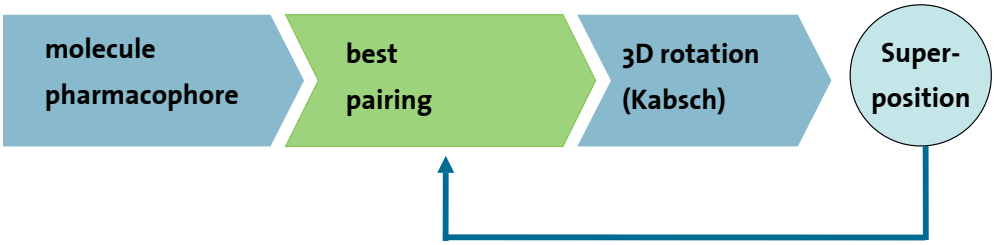
Pharmacophoric Alignment

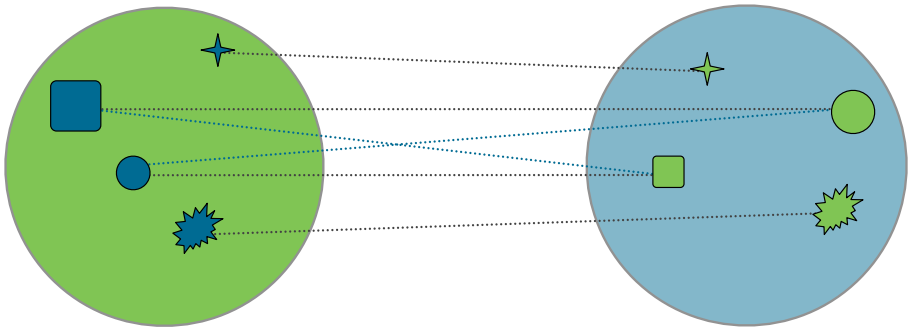


Is pairing valid?

If not, remove invalid pairs and retry

Wolber G. et al., J. Comput.-Aided Mol. Des. 20: 773-388 (2006)

How To Find The Best Pairs ...



Hungarian Matcher (Marrying Problem)

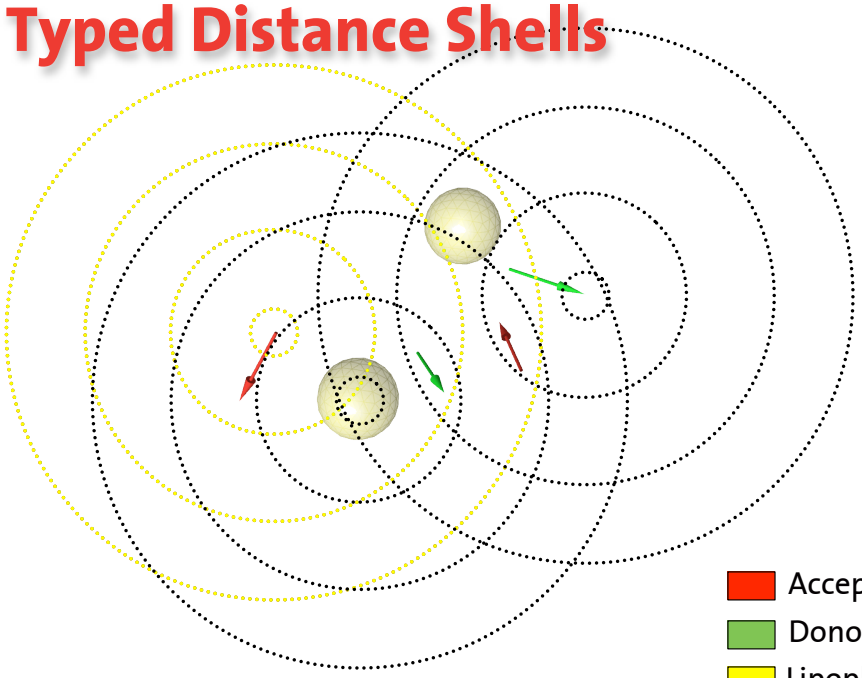
[Edmods 1965]	Matching and a Polyhedron with 0-1 Vertices. J. Res. NBS 69B (1965), 125-30 [nonbipartite application]
[Kuhn 1955]	The Hungarian method for the Assignment Problem. Noval Research Quarterly, 2 (1955) [bipartite variant]
[Richmond 2004]	Application to chemistry: N. Richmond et al. Alignment of 3D molecules using an image recognition algorithm. J. Mol. Graph. Model. 23 (2004) 199-209

Hungarian Matching

- How to define the pharmacophore feature matching cost (similarity)?
  - Use only few feature types
  - Create selectivity by defining geometric relations

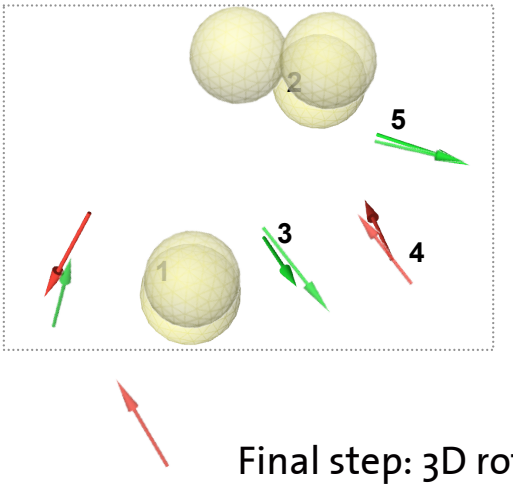
=> Solution: Encode geometry in each feature!

Typed Distance Shells



Distance Characteristics

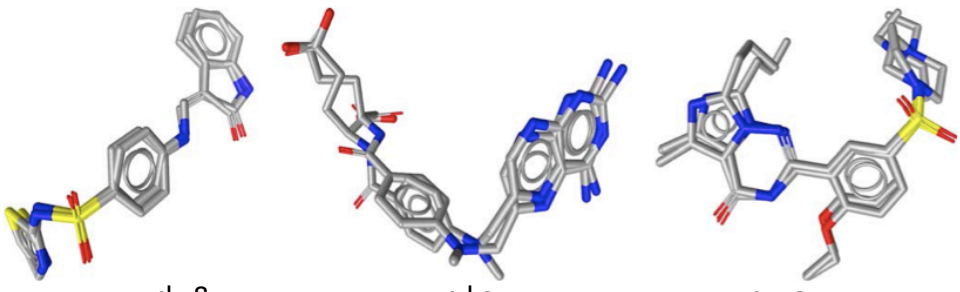
Result: Best matching pairs for each feature



Final step: 3D rotation using Kabsch algorithm

Flexible Alignment

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

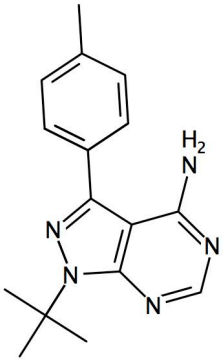


1ke8 1rb3 1xpo

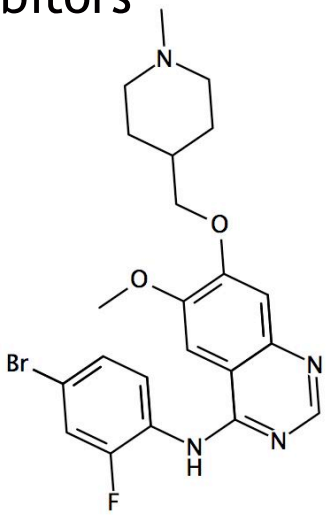
Wolber G. et al., J. Comput.-Aided Mol. Des. 20: 773-388 (2006)

Understand Common Features ...

Example: RET Kinase Inhibitors



2ivv



2ivu

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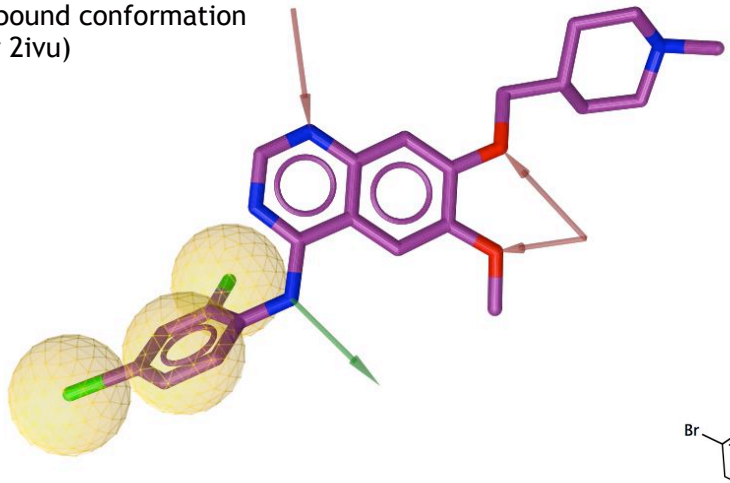
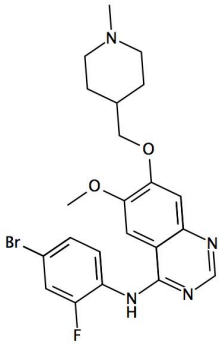
Shared Feature Pharmacophore

RET-Kinase inhibitor
ZD62015, bound conformation
(pdb entry 2ivu)





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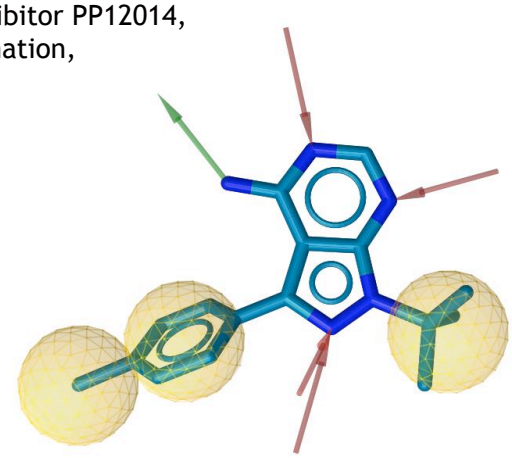
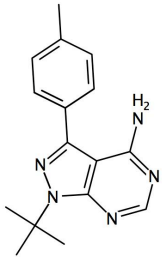
Shared Feature Pharmacophore

RET-Kinase inhibitor PP12014,
bound conformation,
pdb entry 2ivv





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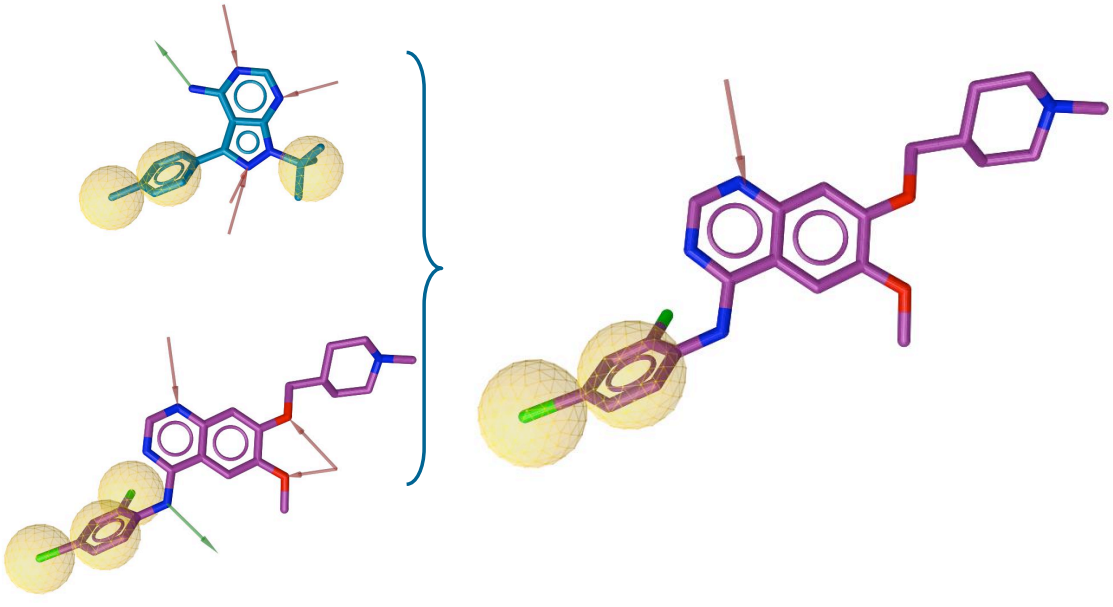
Shared Feature Pharmacophore



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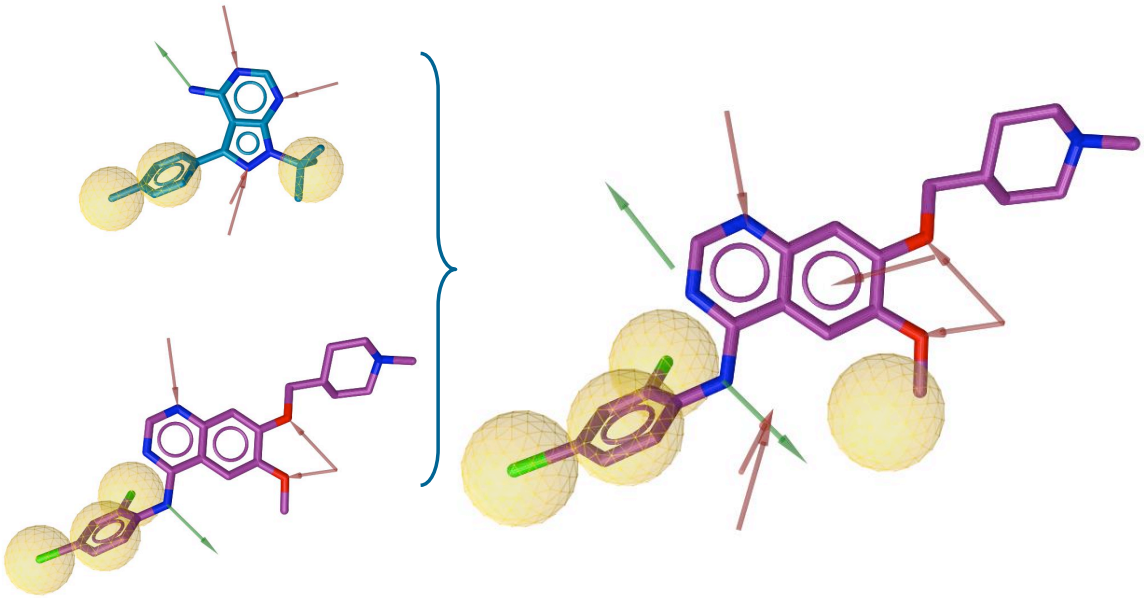
Merged Feature Pharmacophore



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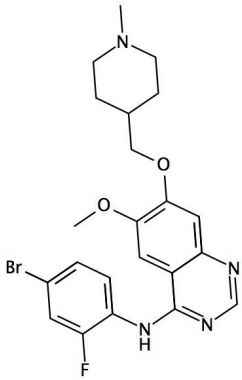
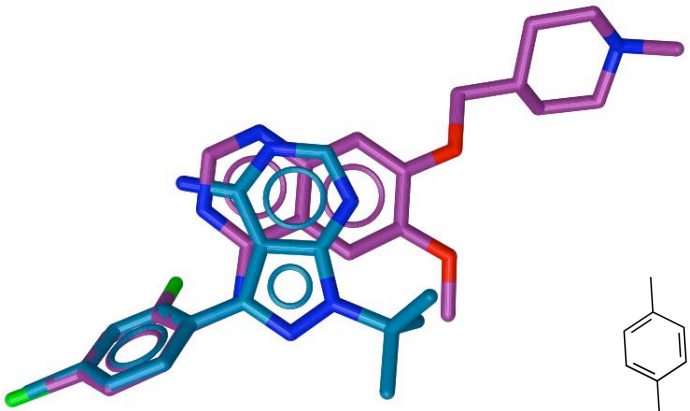
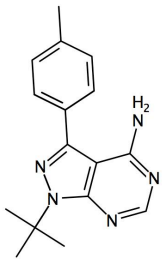
Pharmacophore-based Alignment







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Ligand Profiling Case Study

- 5 viral targets

- 50 pharmacophore models

- 100 antiviral compounds

Will their activity profiles be predicted correctly ?

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Ligand Profiling: Targets

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

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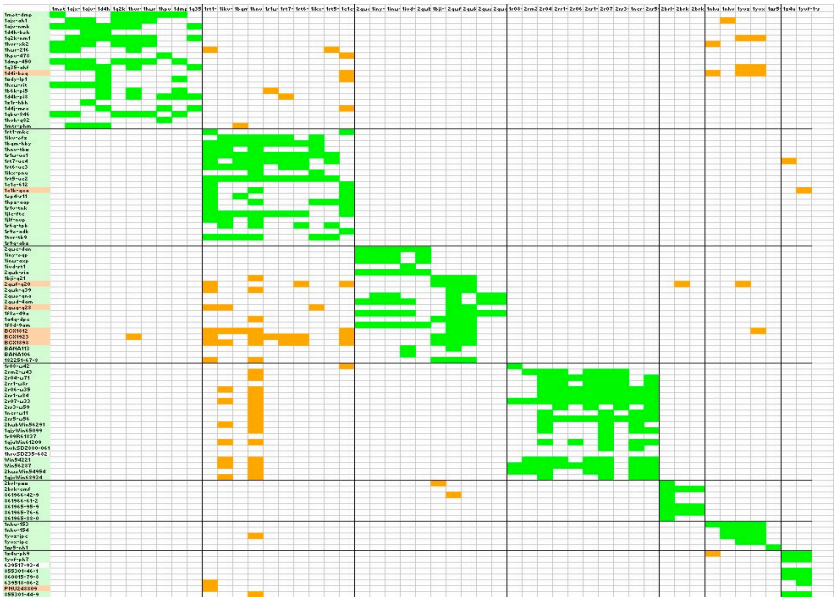
Results Matrix



T. Steindl et al., J. Chem. Inf. Model., 46, 2146-2157 (2006)

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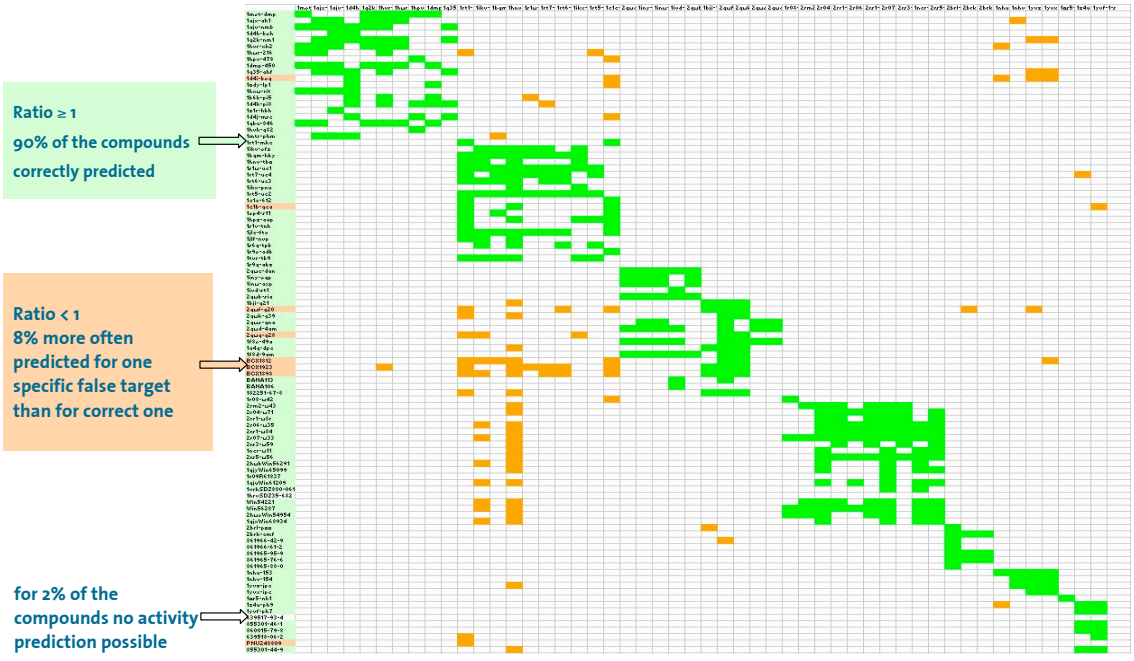
Ligand-directed Analysis



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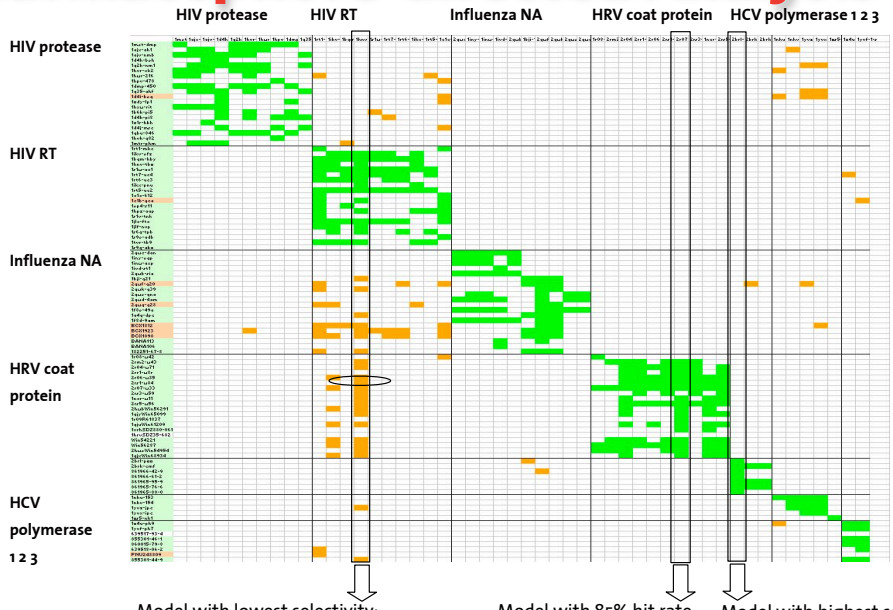
Pharmacophore-directed Analysis



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

Model with 85% hit rate

Model with highest selectivity:
100% of actives (HCV polymerase 1), 100% active and 0% inactive compounds in hit list

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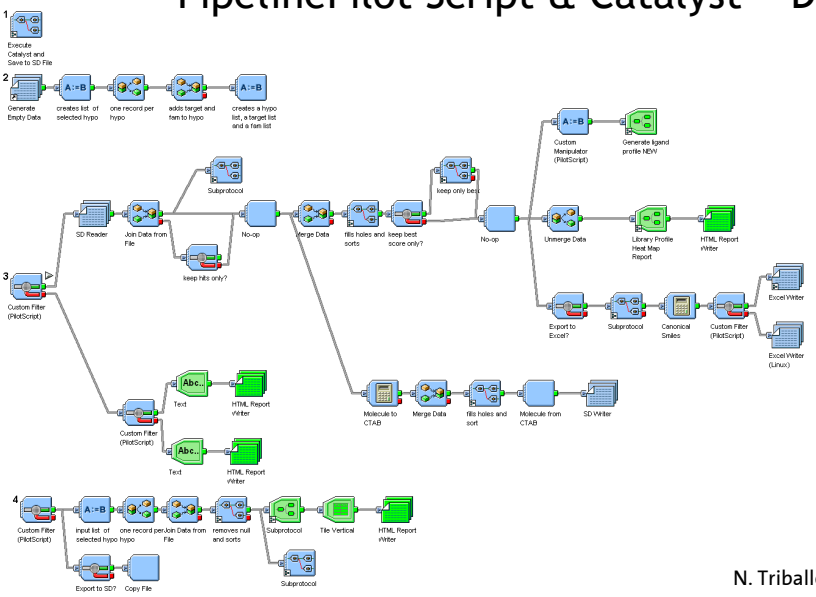
Underlying Screening Platform

PipelinePilot Script & Catalyst™ DB Search



K. Chuang
J. Benedict
N. Triballeau-Hugounencq
Rémy D. Hoffmann

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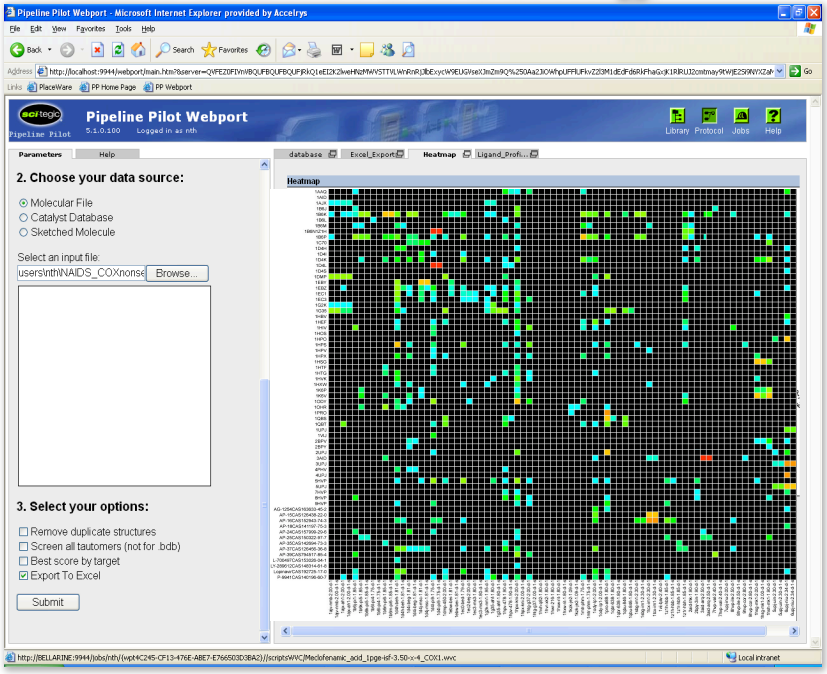
Web Based Parallel Screening



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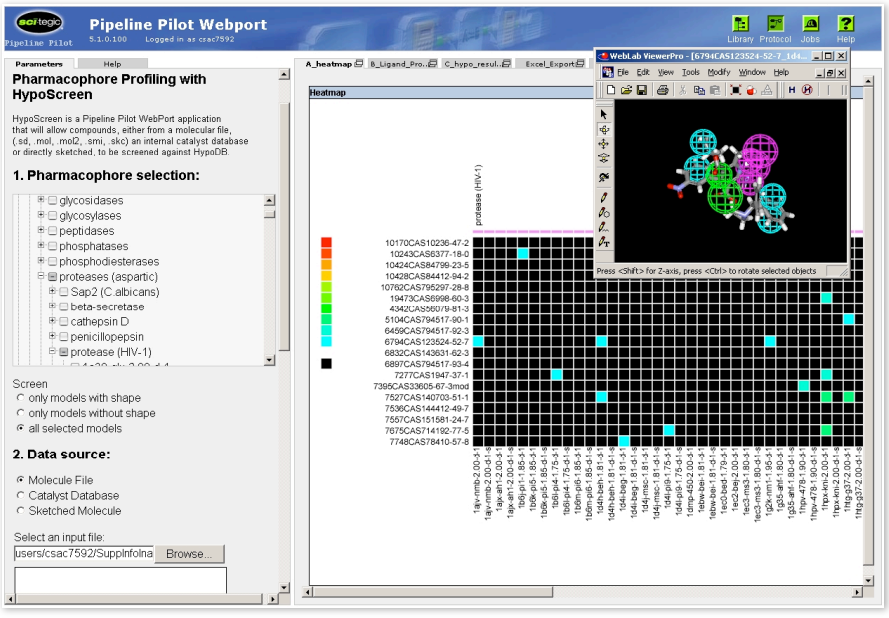
Web Based Parallel Screening



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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 large compound libraries

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Summary ...

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 !

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Inte:Ligand's Pharmacophore Database

~ 300 unique targets ready to use*

- Represented in
  - ~ 200 ligand-based pharmacophore models
  - ~ 2300 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 March 2008

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Conclusions

- SOSA together with parallel pharmacophore-based virtual screening is a straightforward and rapid method for the generation of new lead compounds
- Combined 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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Acknowledgements

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FWF

SIXTH FRAMEWORK PROGRAMME

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Konstantin Poptodorov
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Kareen Chuang

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Alois Dornhofer
Robert Kosara
Thua Huynh Buu Thi Hoang
Thomas Seidel
Gerhard Wolber*

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