University of Silesia, Katowice, Poland
11 – 22 March 2013

# Pharmacophore modeling

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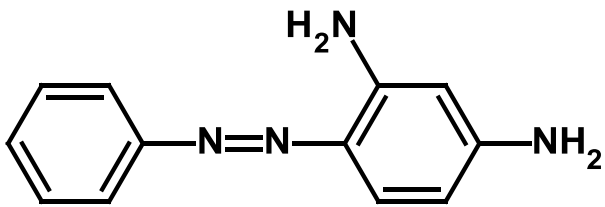
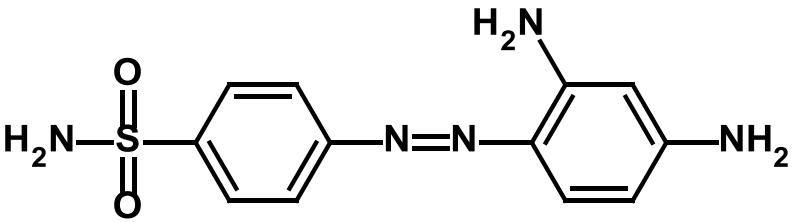
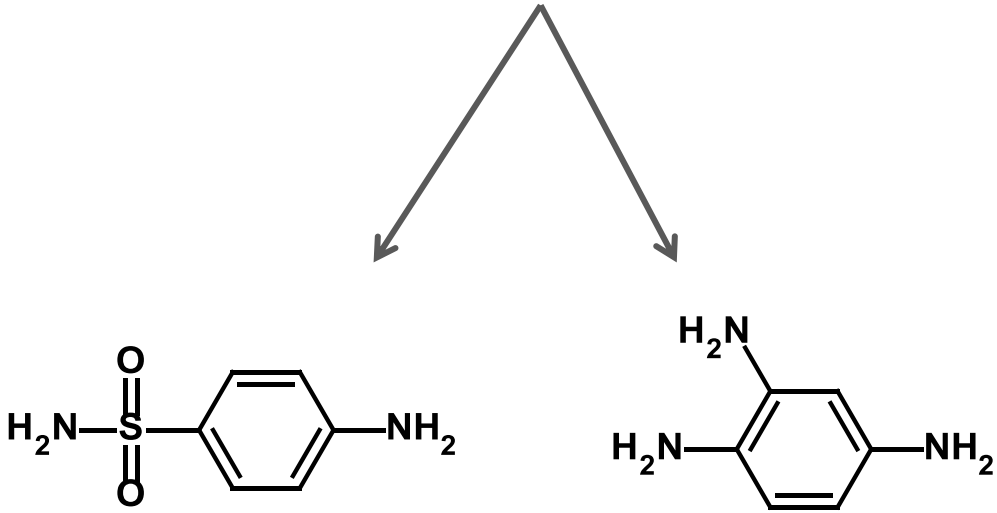
# Early pharmacophore models





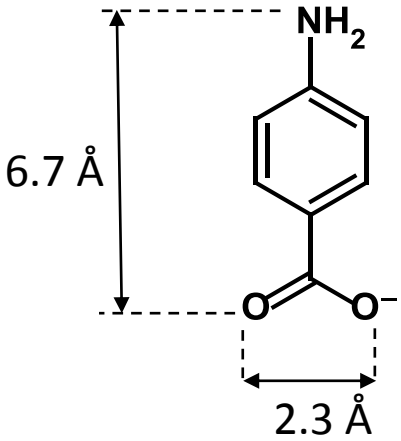



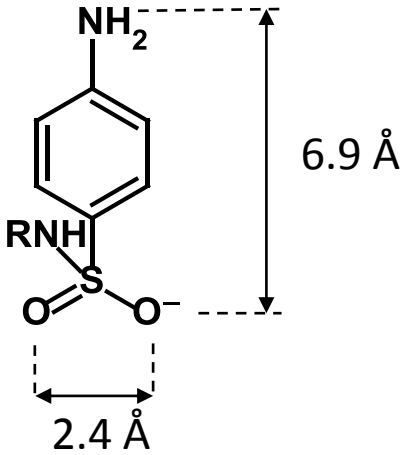

antimicrobal

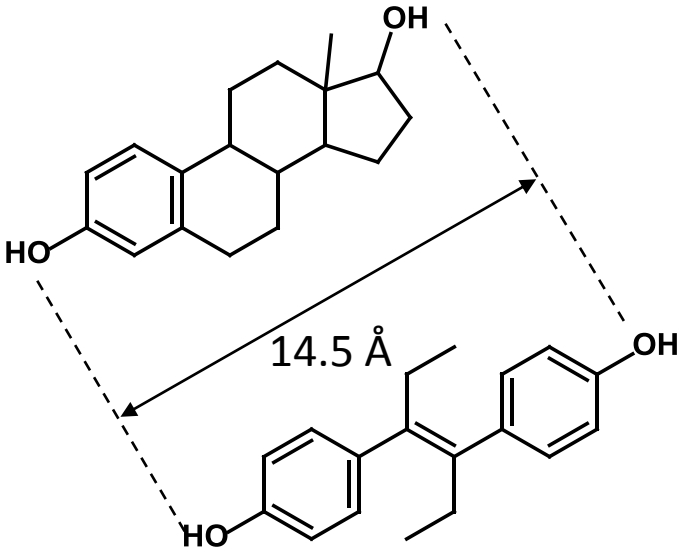
toxic

# Early pharmacophore models





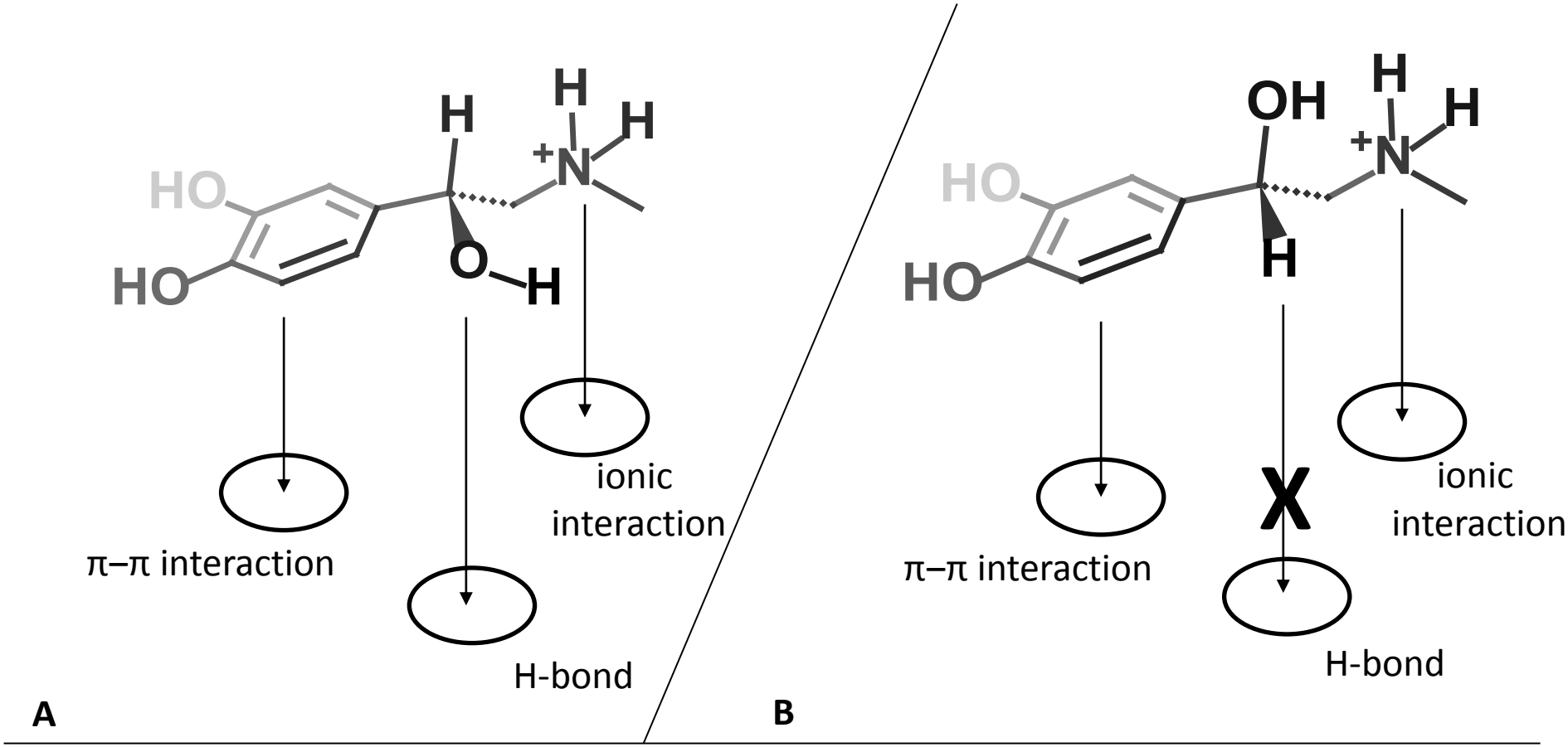




estradiol and *trans*-diethylsilbestrol.

# Early pharmacophore models






(R)-(-) –Epinephrine
(Adrenalin)

(S)-(+) –Epinephrine

# Pharmacophore definition



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

*Annu. Rep. Med. Chem. 1998, 33, 385–395*

# Advantages of 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

# Ligand- and structure-based pharmacophores



## Ligand-based pharmacophore

Exploration of conformational space

Multiple superpositioning experiments

DISCO, Catalyst, Phase, MOE, Galahad, LigandScout ...

## Structure-based pharmacophore

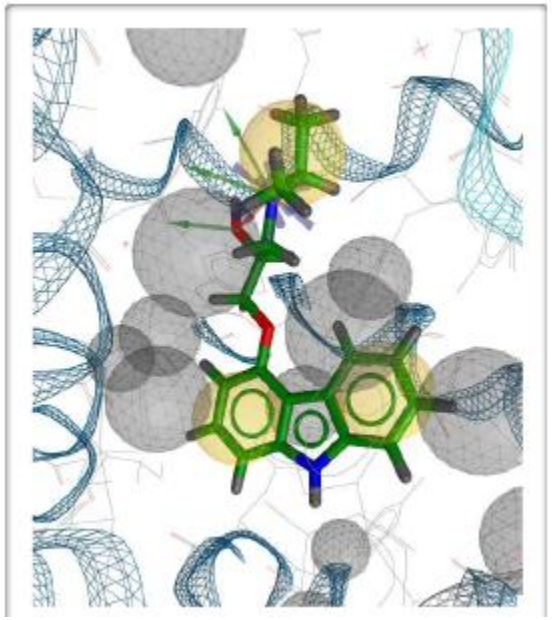
GRID interaction fields: Convert regions of high interaction energy into pharmacophore point locations & constraints
[S. Alcaro et al., Bioinformatics 22, 1456-1463, 2006]

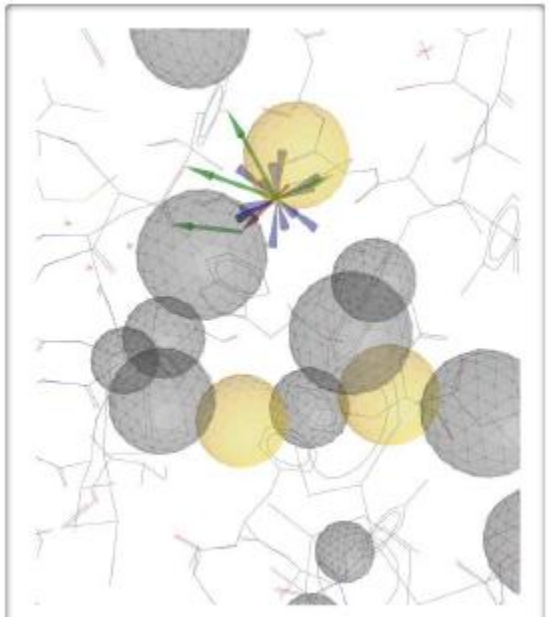
Start from target-ligand complex: Convert interaction pattern into pharmacophore point locations & constraints
[G. Wolber et al., J. Chem. Inf. Model. 45, 160-169, 2005]

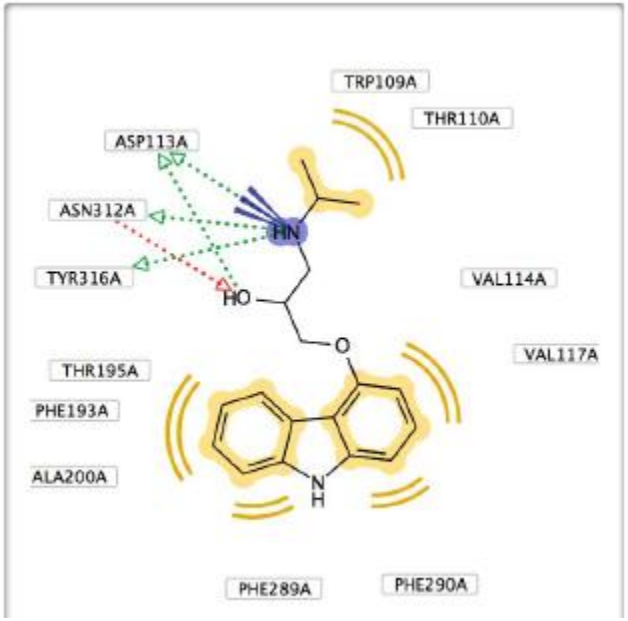
# Feature-based pharmacophore models



Features: Electrostatic interactions, H-bonding, aromatic interactions, hydrophobic regions, coordination to metal ions ...



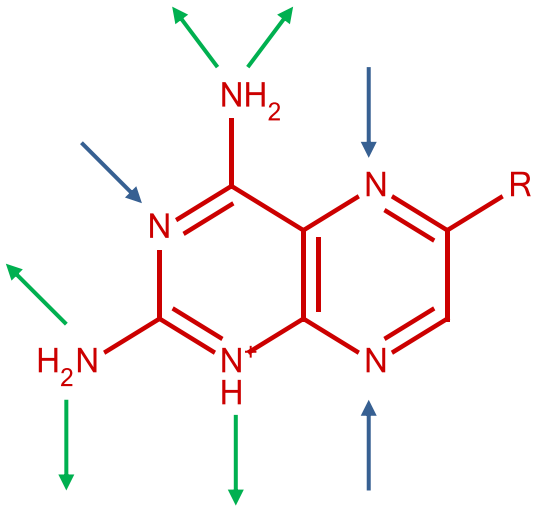




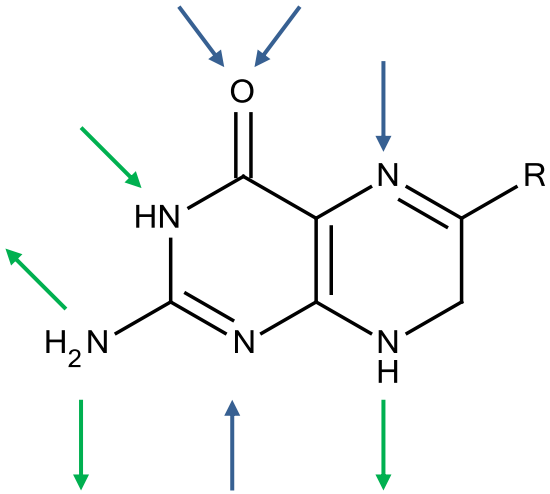
# Atom- and pharmacophore-based alignment



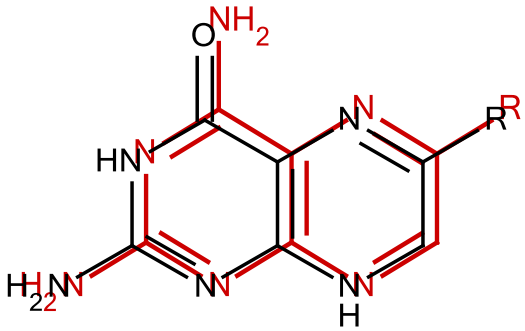
Methotrexate



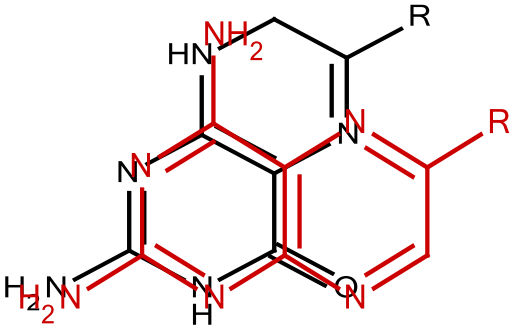

Dihydrofolate




Hydrogen bonding pattern



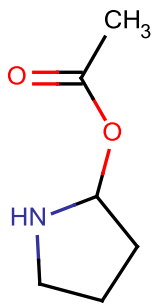

Atom-based alignment

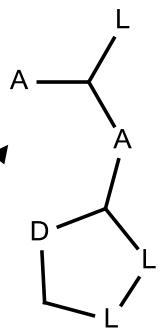



Pharmacophore alignment

# Pharmacophore fingerprints (alignment-free approaches, CATS)

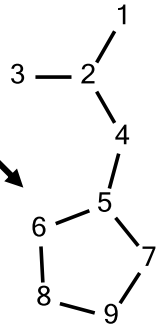





| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | LL | | AL | AL | | DL | LL | | LL |
| 2 | | | | | | | | | |
| 3 | AL | | AA | AA | | DA | AL | | AL |
| 4 | AL | | AA | AA | | DA | AL | | AL |
| 5 | | | | | | | | | |
| 6 | DL | | DA | DA | | DD | DL | | DL |
| 7 | LL | | AL | AL | | DL | LL | | LL |
| 8 | | | | | | | | | |
| 9 | LL | | AL | AL | | DL | LL | | LL |

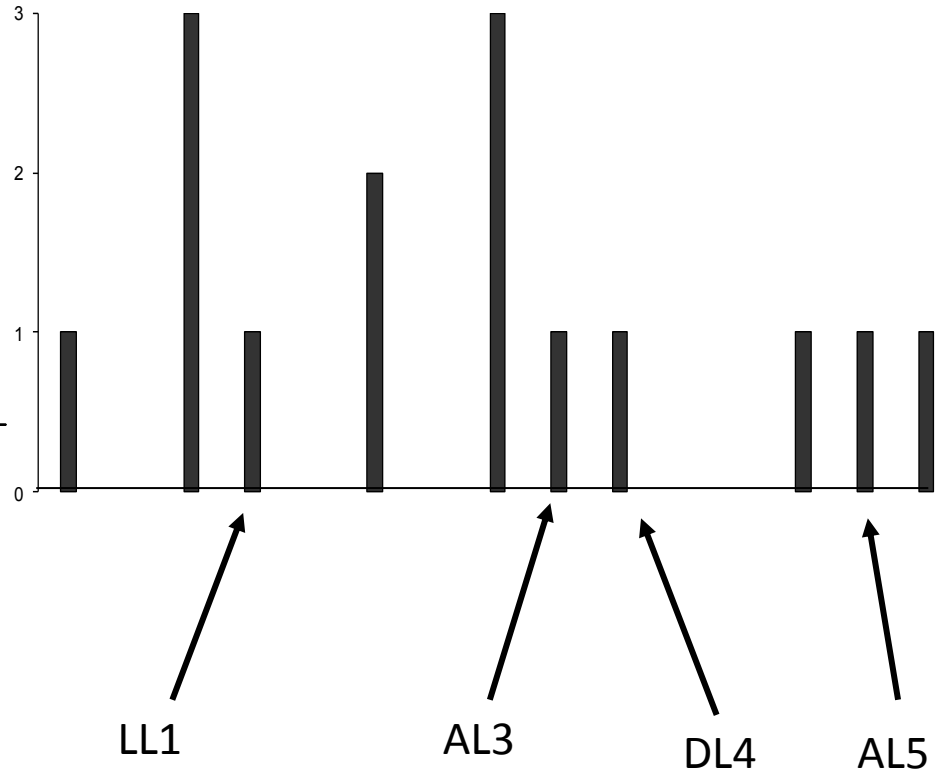
"Pharmacophore matrix"




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 1 | 2 | 2 | 3 | 4 | 4 | 5 | 5 |
| 2 | 1 | 0 | 1 | 1 | 2 | 3 | 3 | 4 | 4 |
| 3 | 2 | 1 | 0 | 2 | 3 | 4 | 4 | 5 | 5 |
| 4 | 2 | 1 | 2 | 0 | 1 | 2 | 2 | 3 | 3 |
| 5 | 3 | 2 | 3 | 1 | 0 | 1 | 1 | 2 | 2 |
| 6 | 4 | 3 | 4 | 2 | 1 | 0 | 2 | 1 | 2 |
| 7 | 4 | 3 | 4 | 2 | 1 | 2 | 0 | 2 | 1 |
| 8 | 5 | 4 | 5 | 3 | 2 | 1 | 2 | 0 | 1 |
| 9 | 5 | 4 | 5 | 3 | 2 | 2 | 1 | 1 | 0 |

Distance matrix

CV={1,0,0,0,0,2,0,0,0,0,0,0,0,0,0,0,3,...,1,0,0,0,0,1}




D – donor; A – acceptor, P – positive;
N – negative, L - lipophilic

# Catalyst

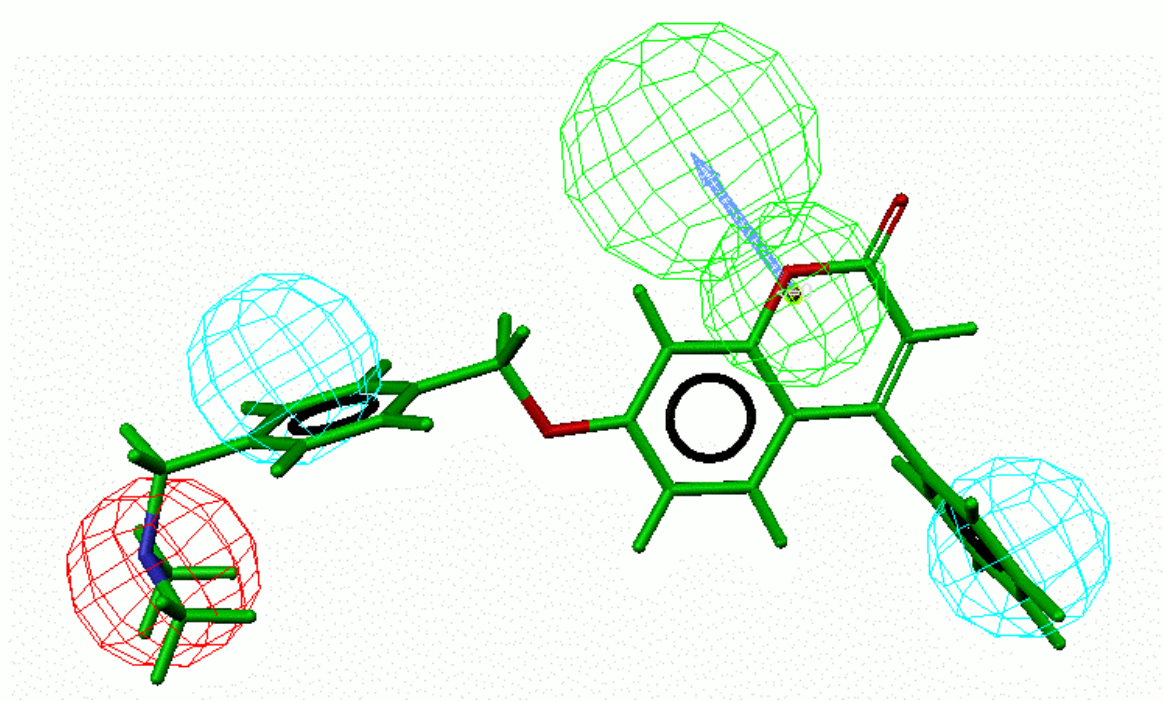


## HipHop

feature-based stepwise alignment

## HypoGen

construction
subtraction
optimization



## HypoRefine

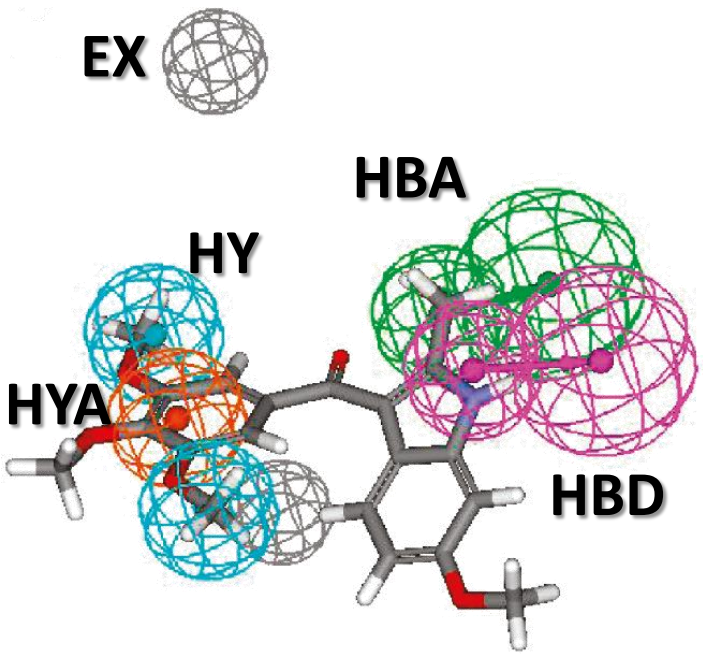
exclusion volumes

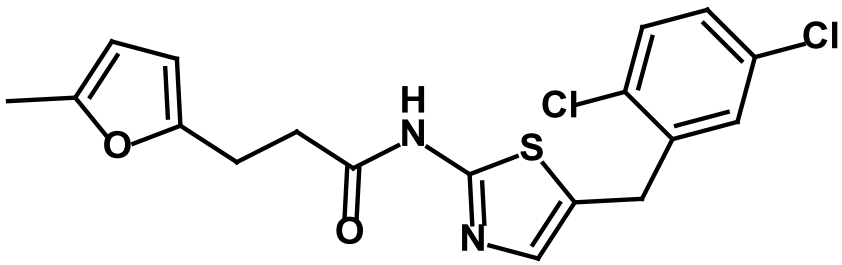
# Catalyst: Example



## Ligand-based model of tubulin inhibitors

21 training compound




| | |
|---|---|
| KB, human oral squamous carcinoma | $IC_{50}$ = 187 nM |
| MCF-7, breast cancer cell line | $IC_{50}$ = 236 nM |
| NCI-H460, human non-small-cell lung cancer cell line | $IC_{50}$ = 285 nM |
| SF-268, human central nervous system cancer cell line | $IC_{50}$ = 319 nM |

Chiang Y.-L. et al, *J. Med. Chem.* 2009, 52, 4221–4233

# PHASE



Clean structures of ligands

Generate conformers

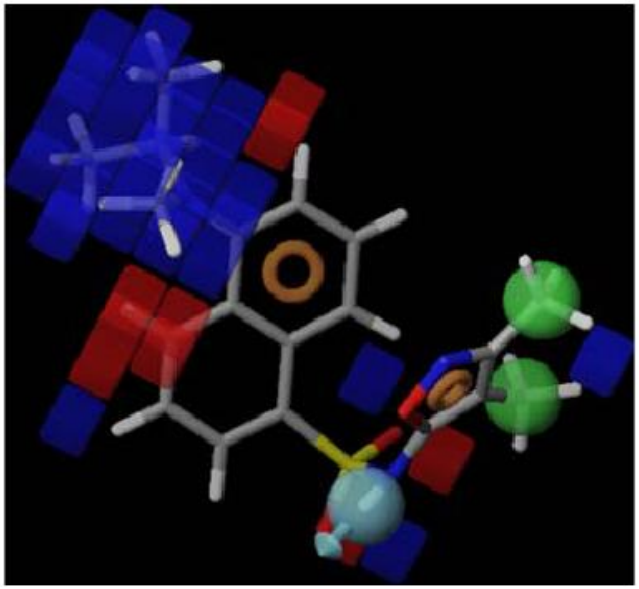
Create pharmacophore sites

Perceive common pharmacophores

Score pharmacophore hypotheses

Build QSAR models

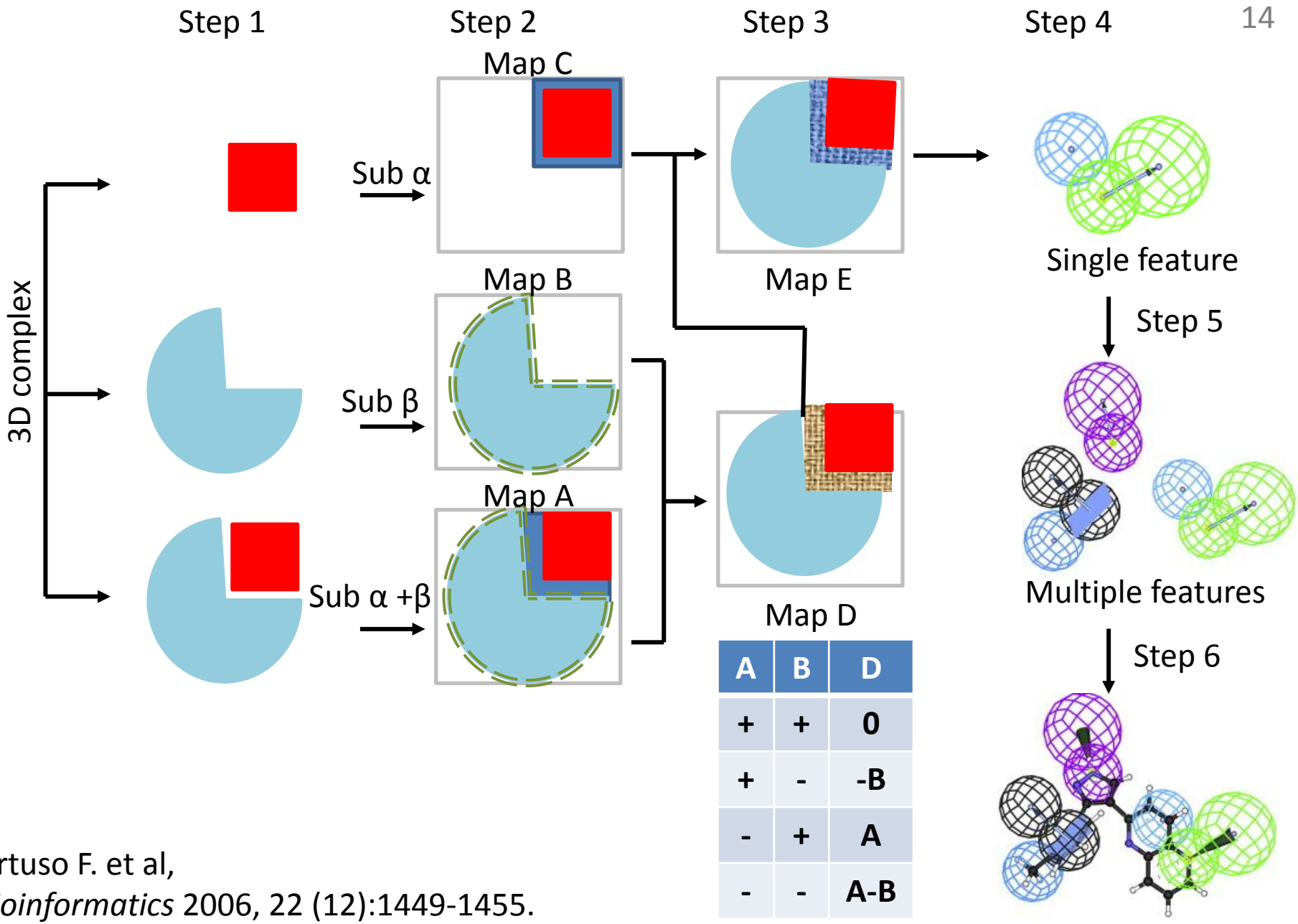
Add excluded volumes



Dixon S.L. et al, *Journal of Computer-Aided Molecular Design*, **2006**, 20, 10-11, pp 647-671

# Grid-based pharmacophore modeling






| A | B | D |
|---|---|---|
| + | + | 0 |
| + | - | -B |
| - | + | A |
| - | - | A-B |

Ortuso F. et al,
*Bioinformatics* 2006, 22 (12):1449-1455.

## Complex preparation

hybridization
connectivity
bond orders
rings closure
etc

## Chemical feature recognition for ligand and protein

remain ligand features which are complementary to the protein features

## Adding exclusion volumes

# LS: Comparability vs Specificity of Chemical Features



| Levels | Universality | Specificity | Classification | Example |
|---|---|---|---|---|
| 1 | -- | +++ | Molecular graph descriptor (atom, bond) with geometric constraint | A phenol group facing a parallel benzenoid system within a distance of 2–4A |
| 2 | - | ++ | Molecular graph descriptor (atom, bond) without geometric constraint | A phenol group |
| 3 | ++ | + | Chemical functionality (hydrogen bond donor, acceptor) with geometric constraint | H-bond acceptor vector including an acceptor point as well as a projected donor point; aromatic ring including a ring plane |
| 4 | +++ | - | Chemical functionality (positive ionizable area, lipophilic contact) without geometric | H-bond acceptor without the projected point; lipophilic group |

# LS chemical features



## SMARTS patterns for chemical features

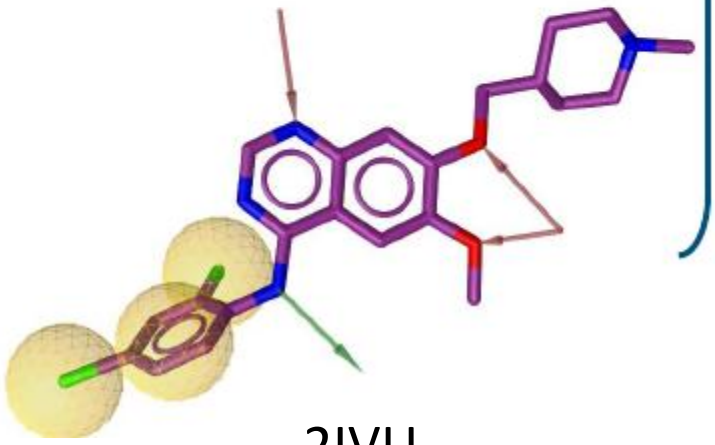
| | Inclusion patterns | Exclusion patterns |
|---|---|---|
| HBA-F | {[O,S]}[#1] | c1nnnn1 |
| | {N}[#1] | |
| | C{F} | |
| HBD | {[N,O,S;X1,X2]} | [–,–2,–3] |
| PI | {[NX3]}([CX4])([CX4,#1])[CX4,#1] | |
| | {N}=[CX3]({[N;H1,H2]})[! N] | |
| | N=[CX3]({[NH1]}){[NH1]} | |
| | {[+,+2,+3;! $(*[–,–2,–3])]} | |
| NI | [S,P](={O})(={O}){[OH]} | |
| | [S,C,P](={O}){[OH]} | |
| | {c}1{n}{n}{n}{n}1 | |
| | {[–,–2,–3;! $(*[+,+2,+3])]} | |

# Consensus of models

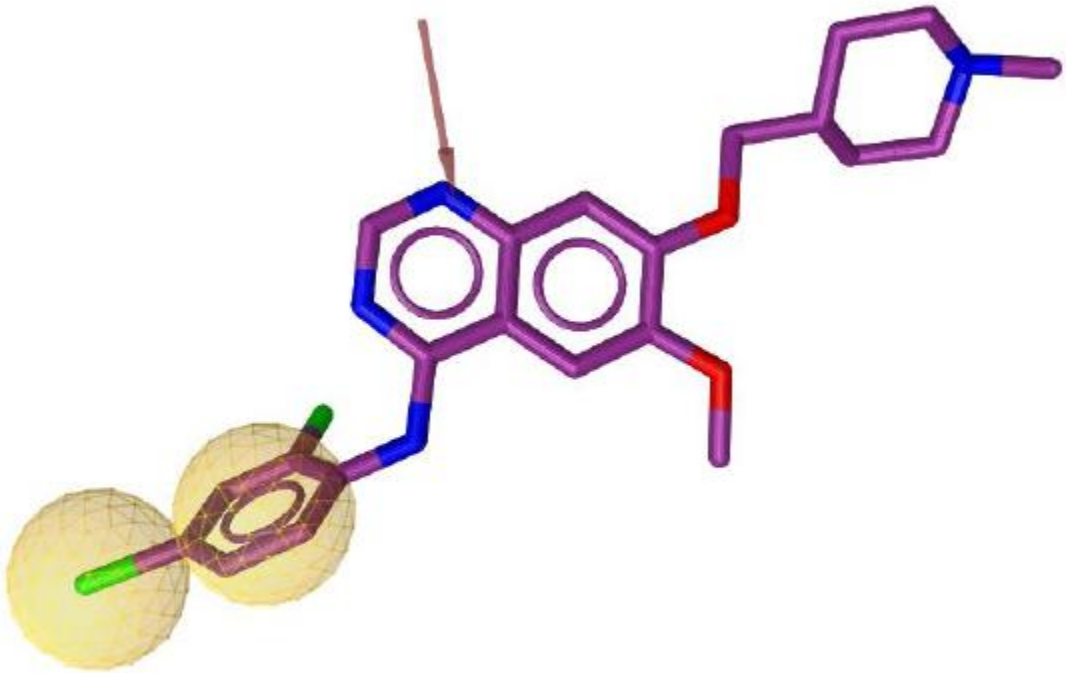


## Shared pharmacophore models

2IVV



2IVU

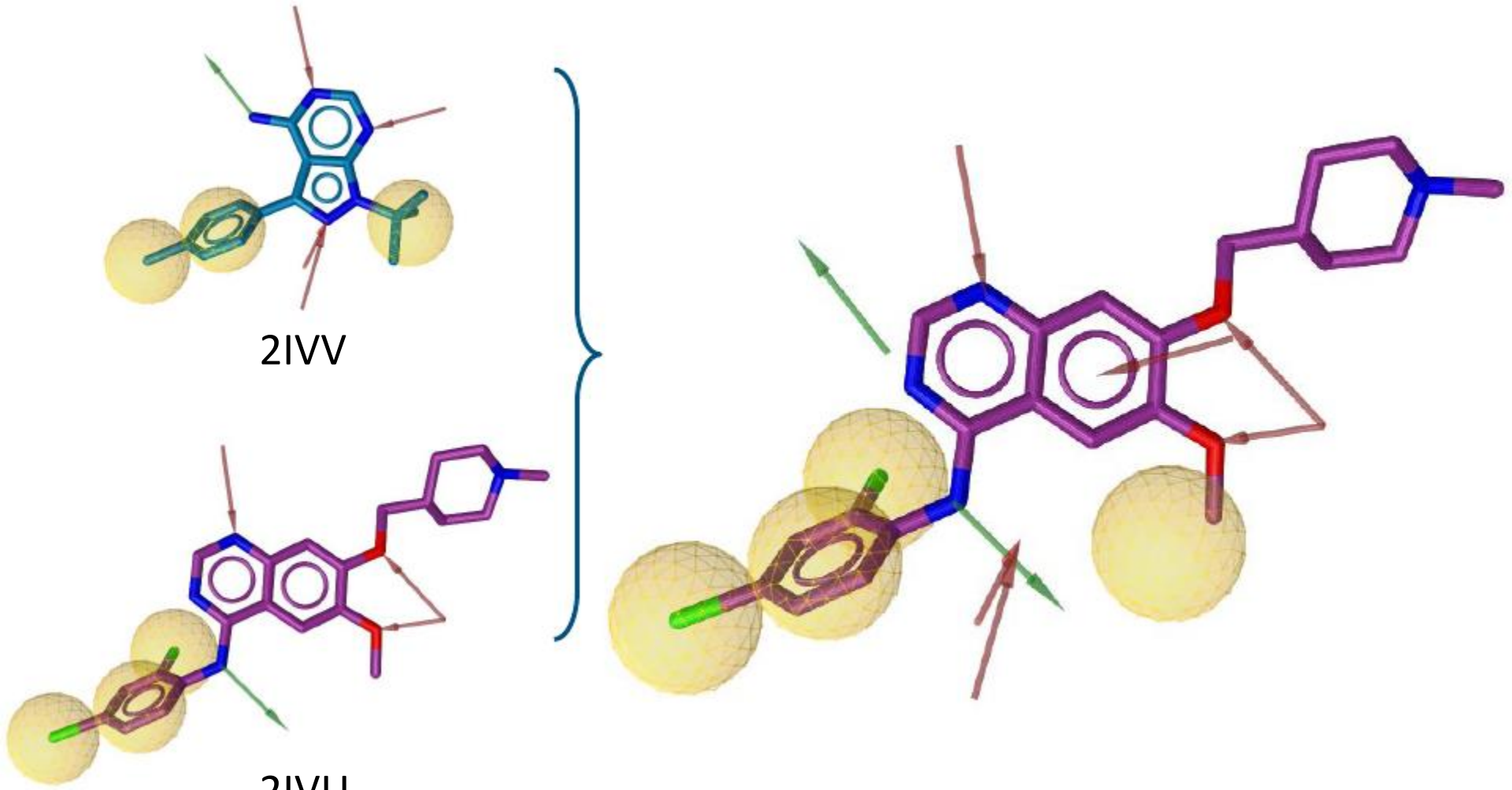


RET Kinase Inhibitors

# Consensus of models



## Merged pharmacophore models




RET Kinase Inhibitors

# Pharmacophore validation



Pharmacophore is an idea – true (ideal) mode of interaction.

Deviation from the true pharmacophore are caused by:

Incomplete datasets for modeling

All H-bonds are of the same strength

Positions of water molecules are considered the same for different ligands

Error are brought from modeling algorithms

That's why the result is called "**pharmacophore model**".

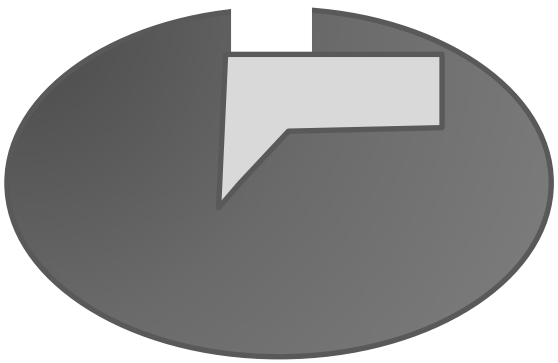
# Pharmacophore validation



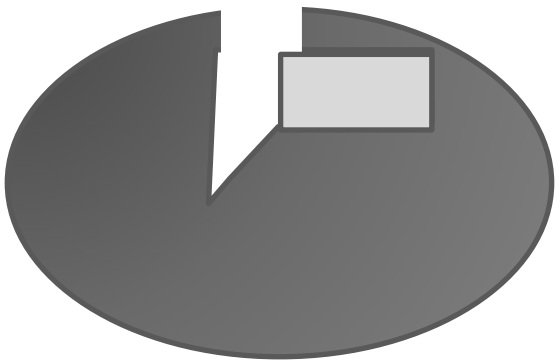
## Ligand-based phamacophore modeling

Internal score functions is not reliable validation

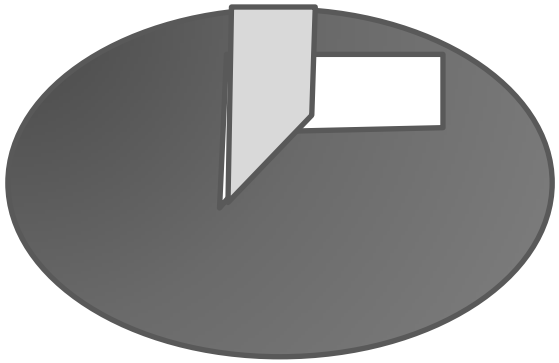
## Structure-based phamacophore modeling



**Substrate**



**Inhibitor 1**

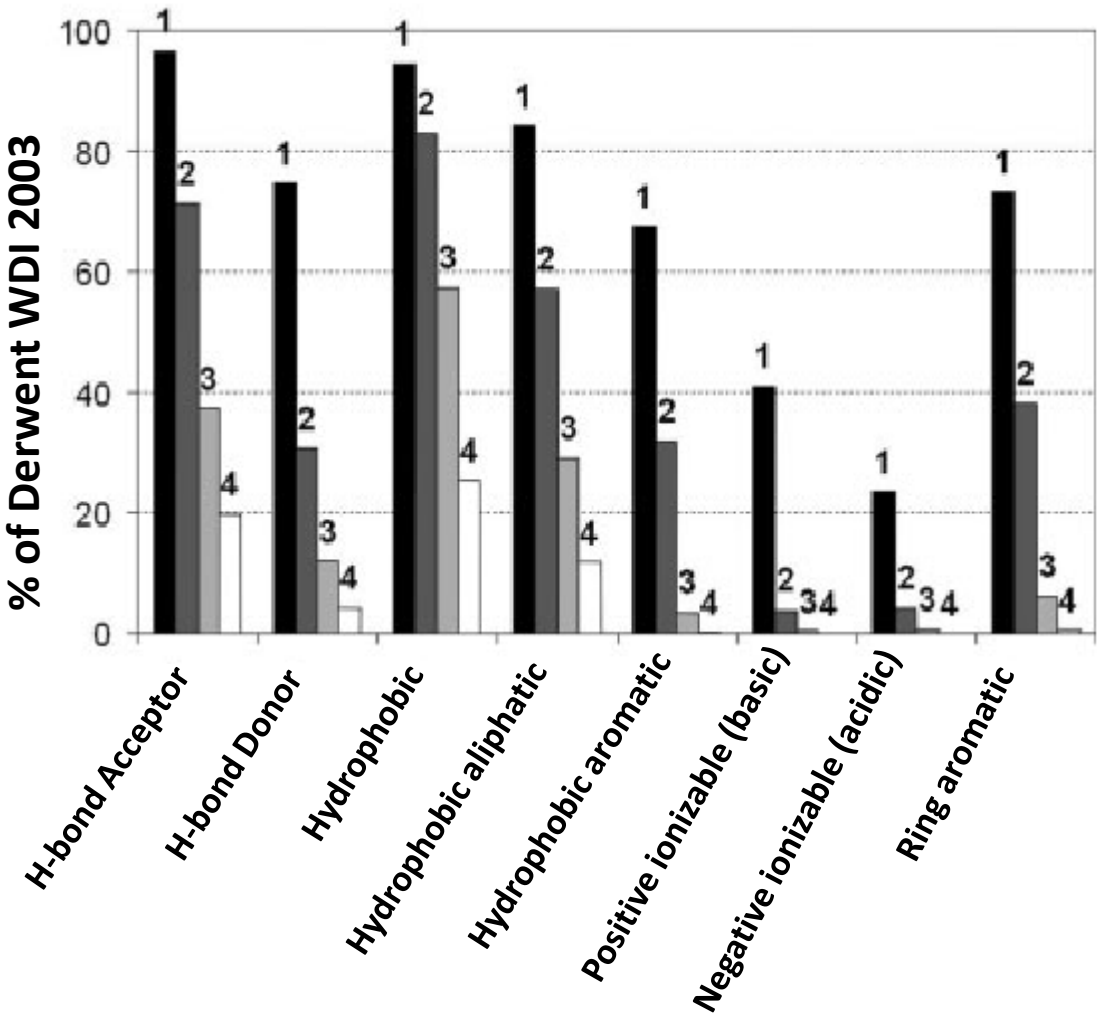


**Inhibitor 2**

# Pharmacophore validation



## Visual inspection



# Pharmacophore validation

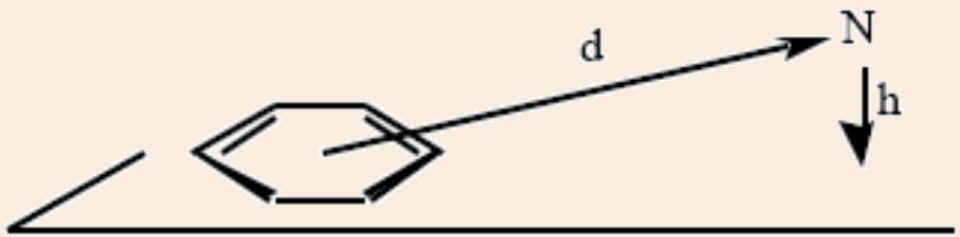


Pharmacophore model should describe known structure-activity relationship (SAR) and explain stereochemistry of ligands

# Comparison with other pharmacophore models

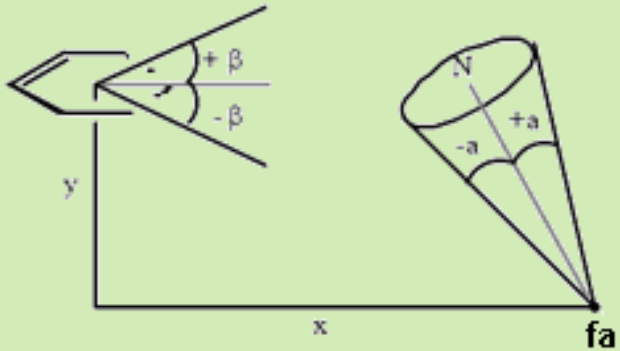


(obtained with different approaches and datasets)




d = 5.2 - 5.6 Å
h = 0.2 - 1.6 Å

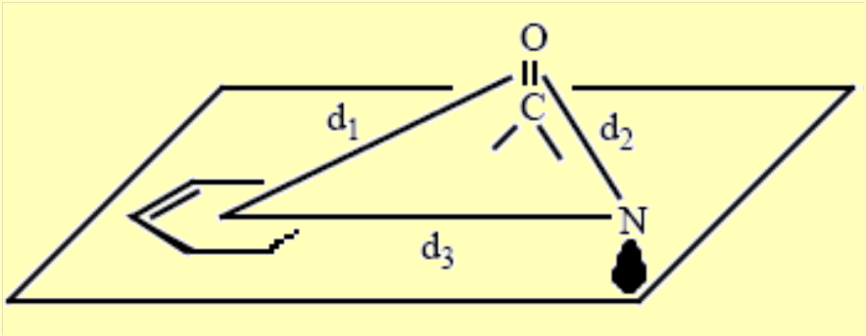
Hibert M.F. et al, Journal of Medicinal Chemistry. 1988. Vol.31. P.1087-1093




x = 5.2..5.7 Å; y = 2.1..2.6 Å

α = -28º..28º; β = -4º..0.4º

Vallgarda C.M.J. et al, Journal of Medicinal Chemistry. 1991. Vol.34. P.497-510




$d_1$ = 7.07Å

$d_2$ = 4.30Å

$d_3$ = 4.88Å

Chilmonczyk Z. et al, Archiv der Pharmazie. 1997. Vol.330. P.146-160

# Pharmacophore validation



## Validation with external test set

| | | Observed | |
|---|---|---|---|
| | | 0 | 1 |
| Predicted | 0 | TN | FN |
| | 1 | FP | TP |

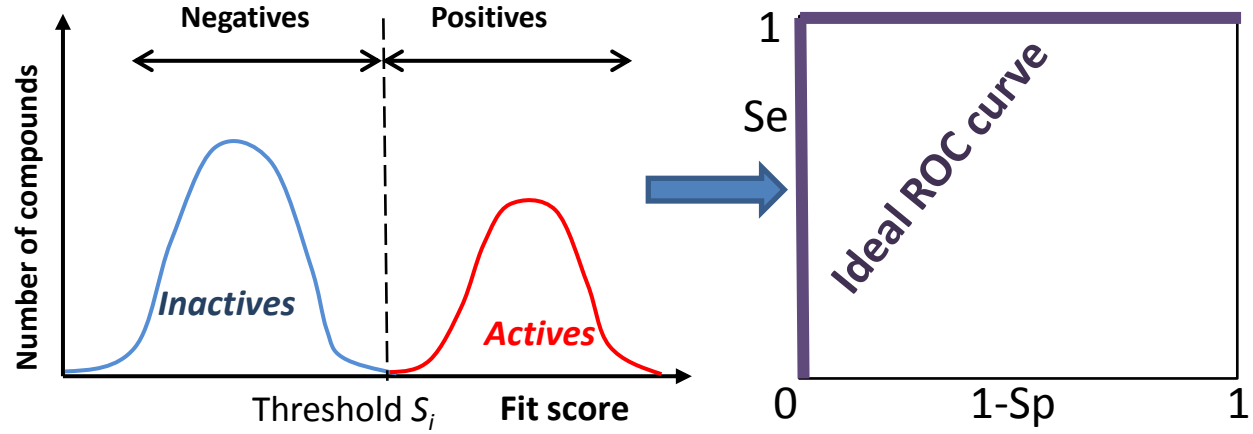
$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}}$$

$$\text{Sensitivity (Recall)} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$

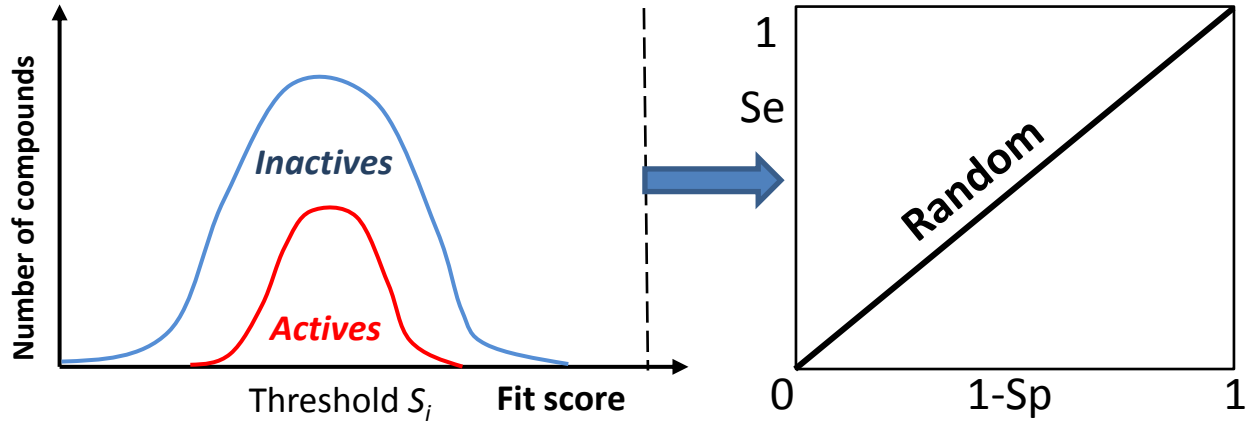
$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}}$$

# Pharmacophore validation: ROC curves

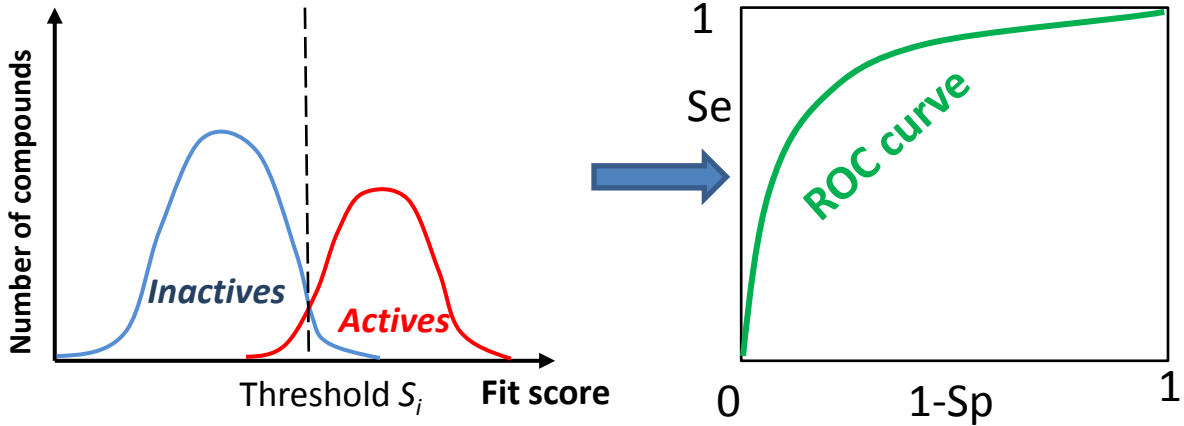





**AUC** = 1




**AUC** =0.5

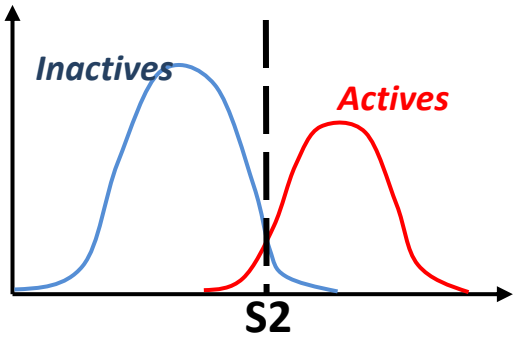



**AUC** = 0-1

# Pharmacophore validation: ROC curves

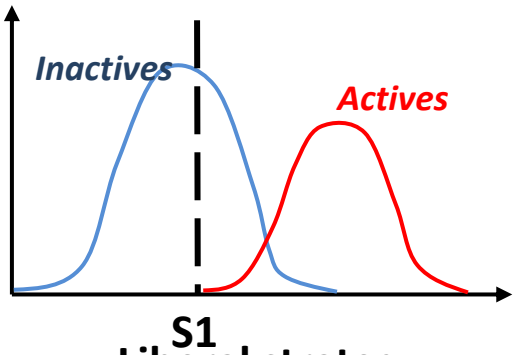


## Decision making from ROC curves




**Break-even strategy**

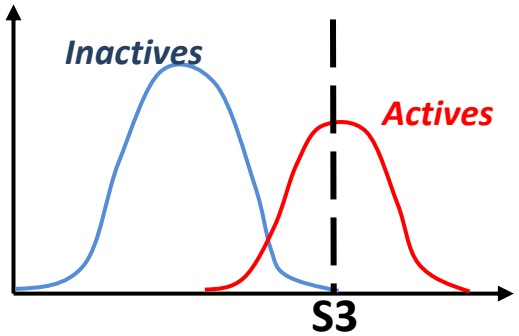
Optimize both Se and Sp




**Liberal strategy**

Increase chemical diversity

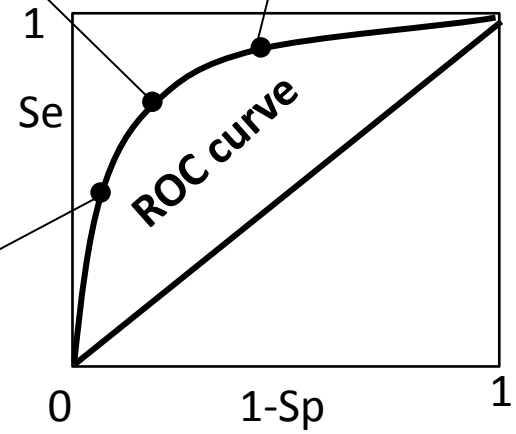
Innovative




**Conservative strategy**

Increase the hit rate

Faster, cheaper, motivating



# Pharmacophore: Ligand profiling



| 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 | HIV infection, AIDS | Synthesis of a virion DNA, integration into host DNA and transcription | Inhibition at allosteric site |
| Influenza virus neuraminidase | 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 |
| Hepatitis C virus (HCV) RNA polymerase | Hepatitis C | Viral replication, transcription of genomic RNA | Inhibition at various allosteric sites |

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

# Pharmacophore: Ligand profiling



5 targets

100 ligands

50 complexes and

50 pharmacophore models

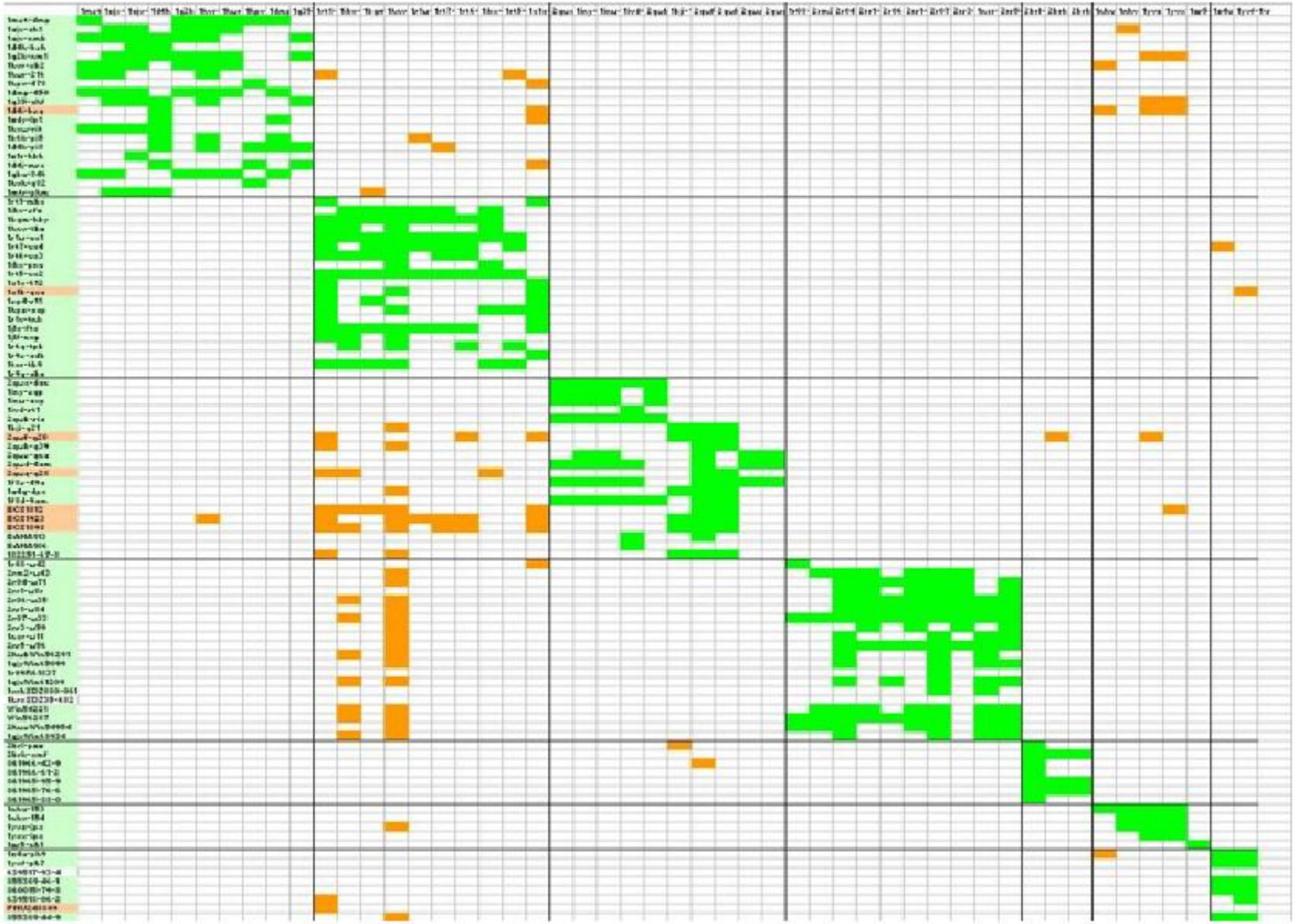
T. Steindl et al., *J. Chem. Inf. Model.*, 46, 2146-2157 (2006)

# Pharmacophore: Ligand profiling



## Pharmacophore models

Ligands



# Pharmacophore: Ligand profiling

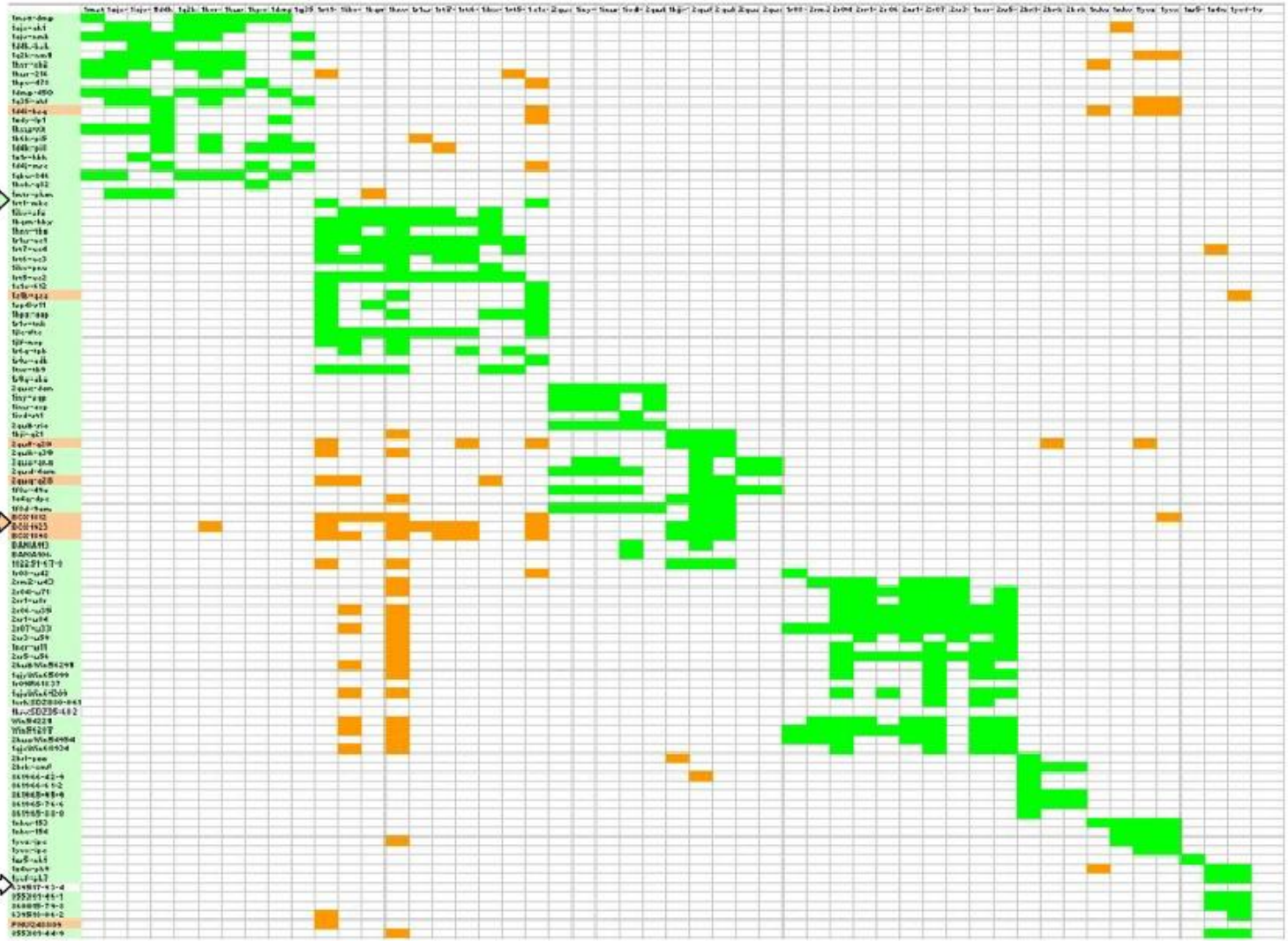


## Ligand-directed analysis

Ratio ≥ 1
90% of the compounds correctly predicted

Ratio < 1
8% more often predicted for one specific false target than for correct one

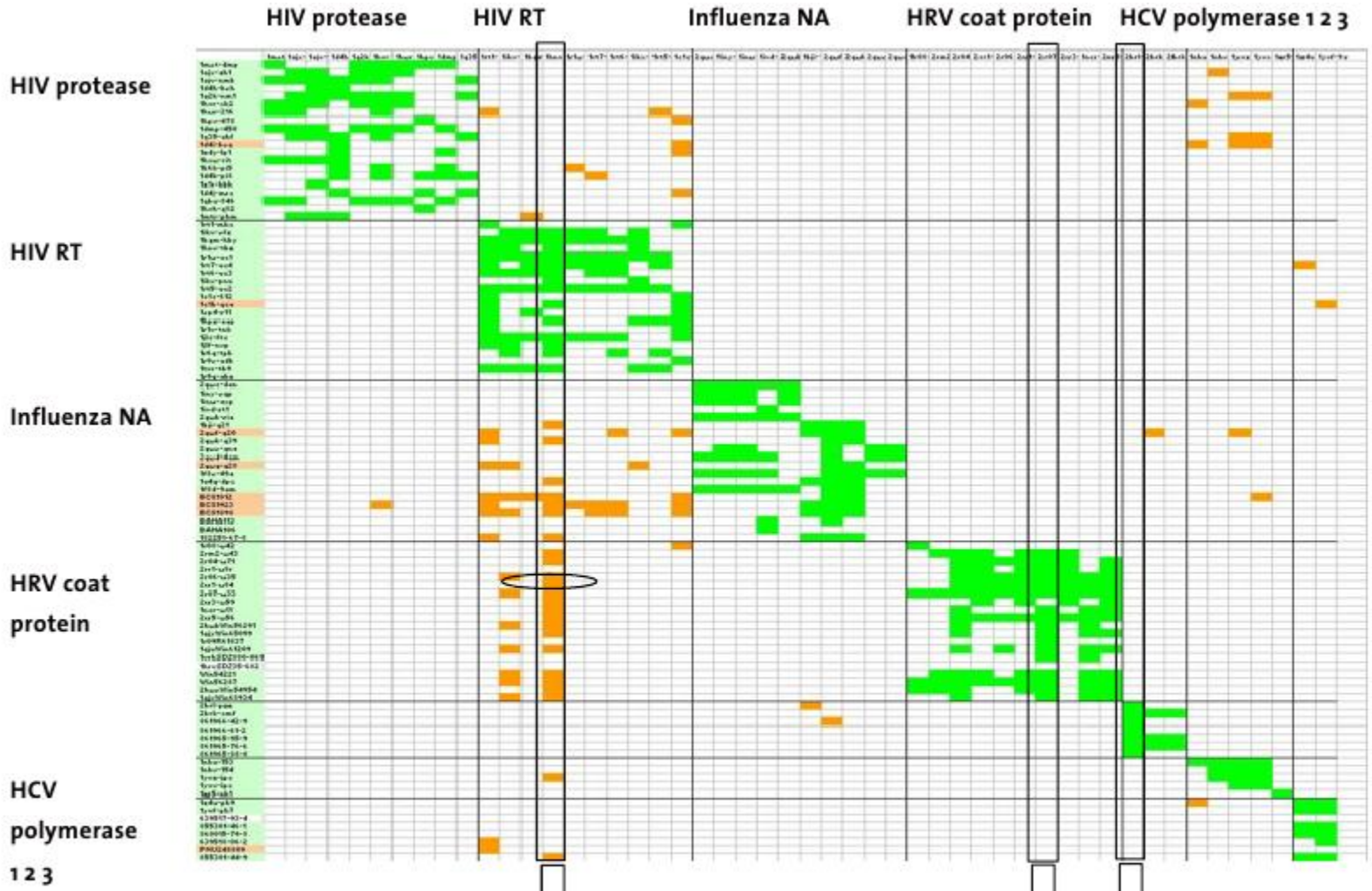
for 2% of the compounds no activity prediction possible



# Pharmacophore: Ligand profiling

## Pharmacophore-directed analysis





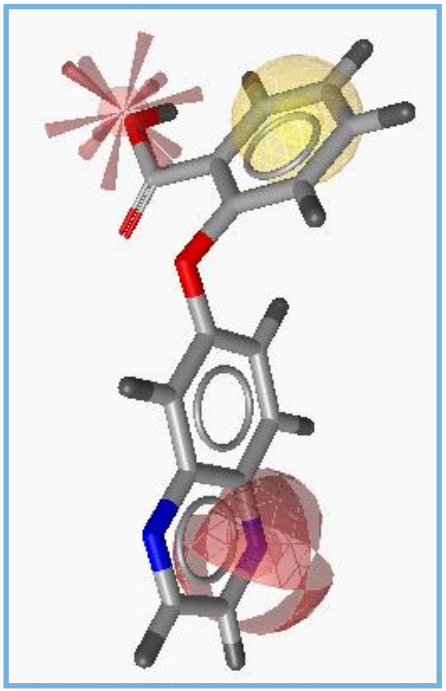

Model with lowest selectivity:
70% of actives (HIV RT), but 75% from one specific false target (HRV coat protein)
40% active and 60% inactive compounds in hit list

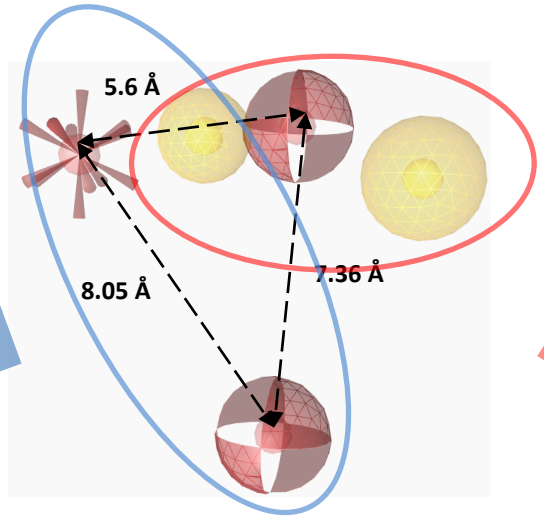
Model with 85% hit rate

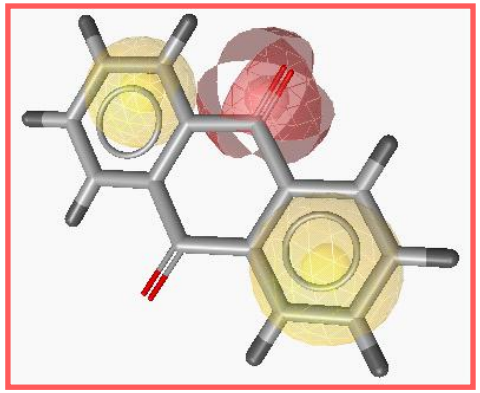
Model with highest selectivity:
100% of actives (HCV polymerase 1),
100% active and 0% inactive compounds in hit list

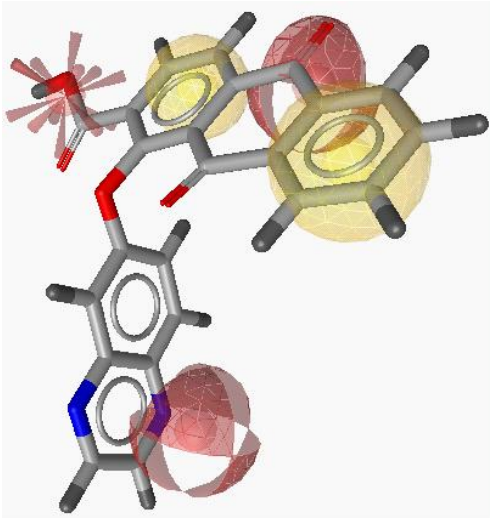
# Library design by fragment-based pharmacophore approach









# Common problems of pharmacophore



Choosing of the training set compounds (ligand-based) or complexes (structure-based)

Ligand-based:
generation of conformers, choosing of “bioactive” conformer and reference molecule

Structure-based:
different interaction patterns of different ligands; high specificity of models

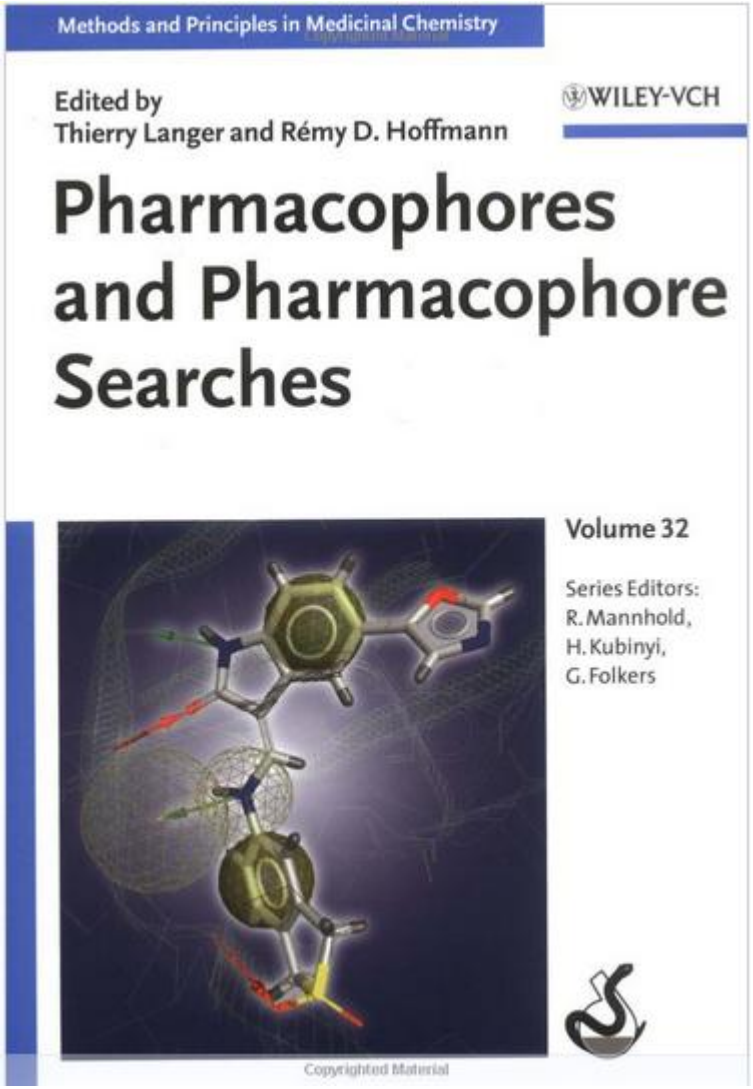
# Recommended literature



**Pharmacophores and Pharmacophore Searches**

Eds.: Thierry Langer, Rémy D. Hoffmann

2006



# Recommended literature



JOURNAL OF CHEMICAL INFORMATION AND MODELING

Article

pubs.acs.org/jcim

## Comparative Analysis of Pharmacophore Screening Tools

Marijn P. A. Sanders,[†,#] Arménio J. M. Barbosa,[‡] Barbara Zarzycka,[§] Gerry A.F. Nicolaes,[§] Jan P.G. Klomp,[∥] Jacob de Vlieg,[†,⊥] and Alberto Del Rio*[,‡]

*J. Chem. Inf. Model.*, **2012**, 52 (6), pp 1607–1620