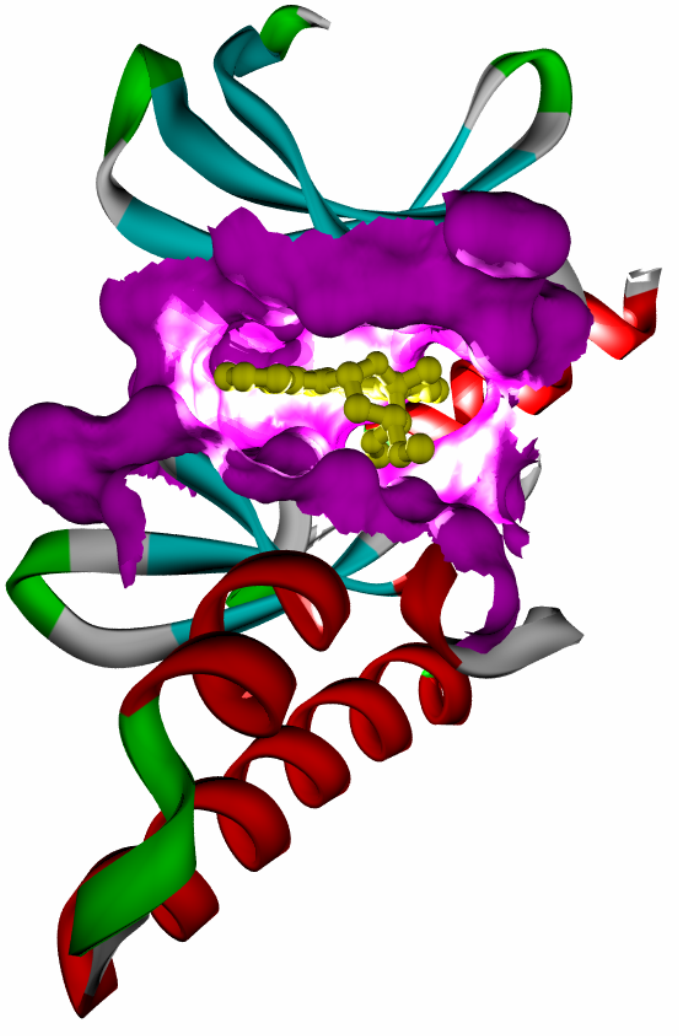
Applications of Virtual Screening using High-Throughput Docking



Basel Computational Biology Conference 2004

E. Vangrevelinghe,
Molecular & Library Informatics,
Novartis Institutes for BioMedical Research,
Basel, Switzerland

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Introduction

- **Advances in genomics have resulted in a large increase in the number of potential therapeutic targets**

- **Hundred fold increase in biological screening data did not lead to correspondent increase in productivity (New Chemical Entities)**

  - **Need for new concepts and techniques to balance quantity and quality**

  - ***In silico* screening, and in particular High-Throughput Docking (HTD), can be used to optimize screening performance and to identify lead candidates among large collections of 3D molecular structures**

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Definitions

in silico Screening

"The selection of compounds by the evaluation of their desirability in a computational model."

High Throughput Docking (HTD)

"A computational process that predicts quickly the binding mode and estimates binding affinity of a large number of ligands to a target receptor."

HTD workflow 1/2

Database of 3D structures (corporate, commercial or virtual)

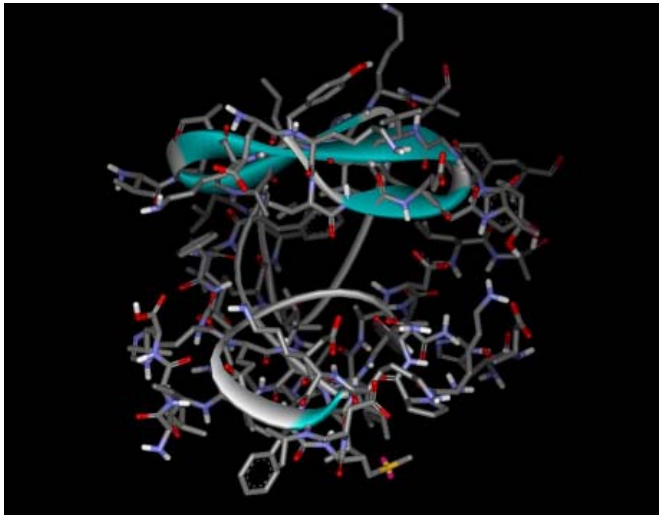
3D coordinates generation protonation states

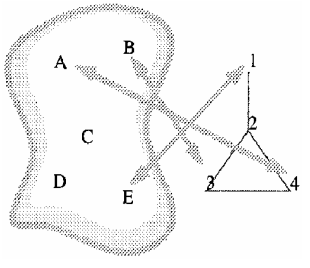
Docking software (Glide, Gold, FlexX,…)

Automatically docks (orients/fits) a series of 3D ligands into an active site

3D model of the selected Target (X-ray from PDB or homology model)

Binding site need to be identified





Searching
- Torsional
- Rotational
- Translational

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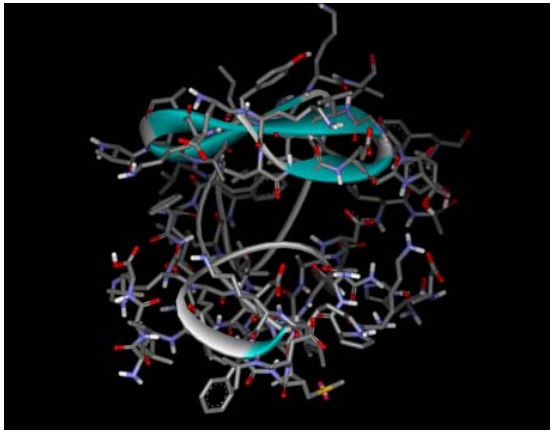
HTD workflow 2/2



Scoring function
Force field (DOCK)
Empirical (Chemscore)
Knowledge (Drugscore)

- **Hydrogen Bond**
- **Ionic Interaction**
- **Hydrophobic Interaction**
- **Entropy Terms**

A scoring function is used to quantitatively rank the ligands according to binding affinity

Hits List

Best scored compounds

Compound selection / Biochemical assays

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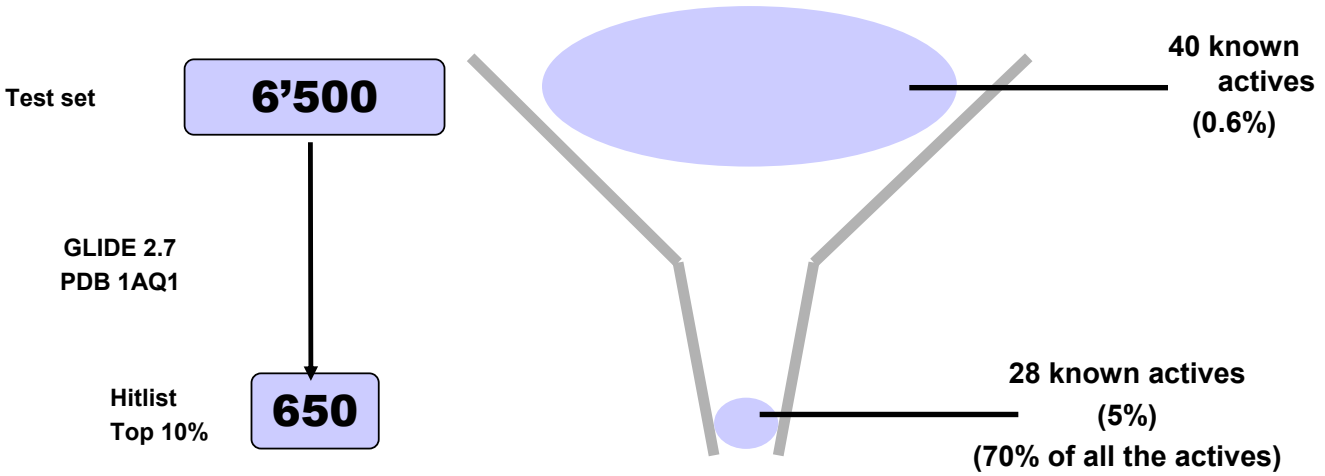
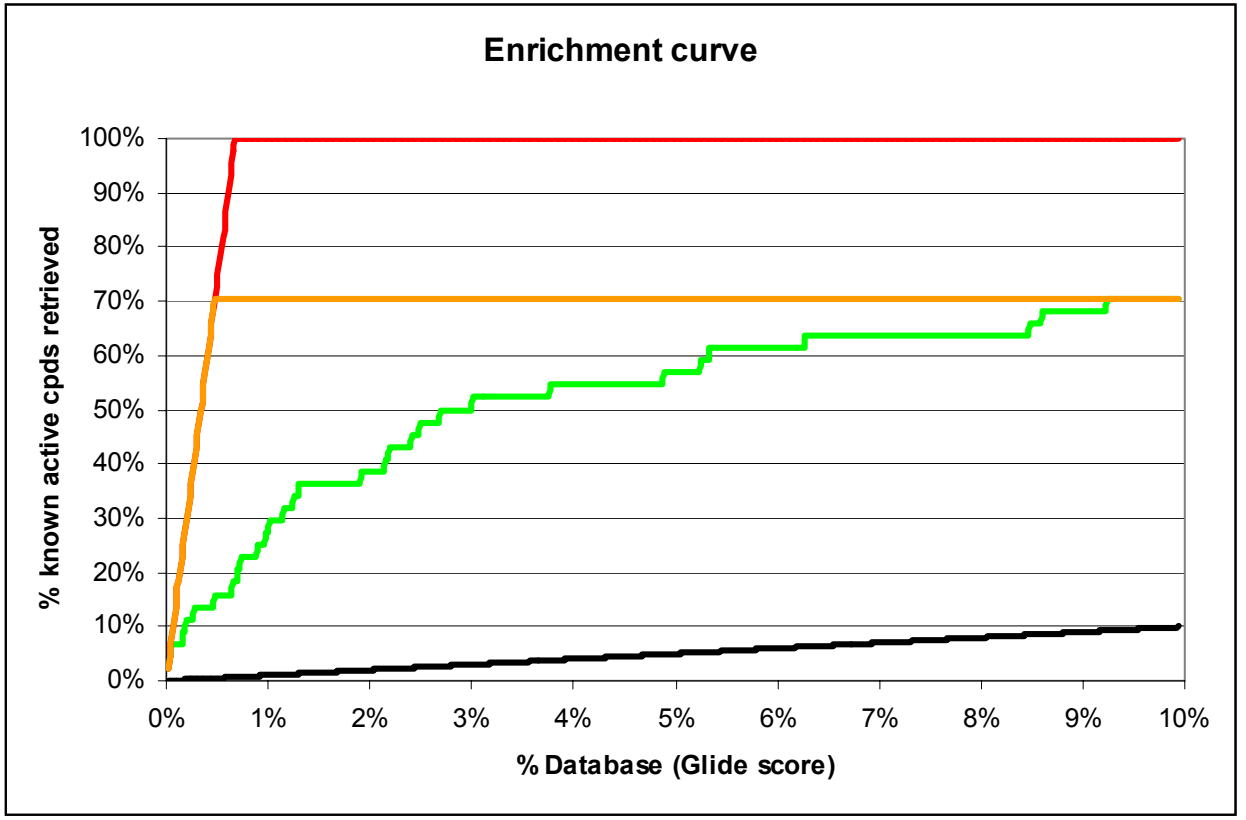
Example

- **Test case on CDK2**
  - **6'460 compounds (randomly selected from ACD)**
  - **40 known active compounds (chemically diverse)**



Example



Enrichment factor is 20 in the Top 2%

E. Vangrevelinghe, [BC]^2, Basel, March 2004

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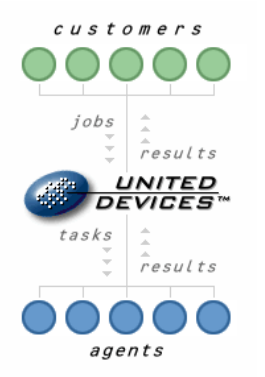
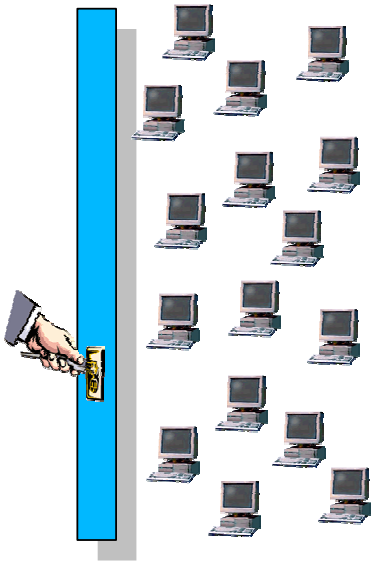
Recent improvements

- "Relatively" new technology (DOCK by Kuntz *et al* in 1982)
  - more accurate algorithms
  - Improvements in the accuracy of the scoring functions
  - Dramatic increases in computer power

Grid Computing

- most desktop CPUs are idle most of the time
- aggregate power of these idle resources





Linux Cluster

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Limitations of the HTD method

- **3D structure of the target**
  - **X-ray model or good homology model (may be validated by docking)**
- **Rigid receptor assumption**
  - **Possibility to use several different conformations of the binding site**
- **Scoring function weaknesses**
  - **Possibility to use several scoring functions**
  - **Targets with a flat binding pocket are more difficult**
  - **knowledge**
- **HTD hits need to be confirmed**
  - **A binding assay should be available**
  - **And/or NMR, MS, X-ray**

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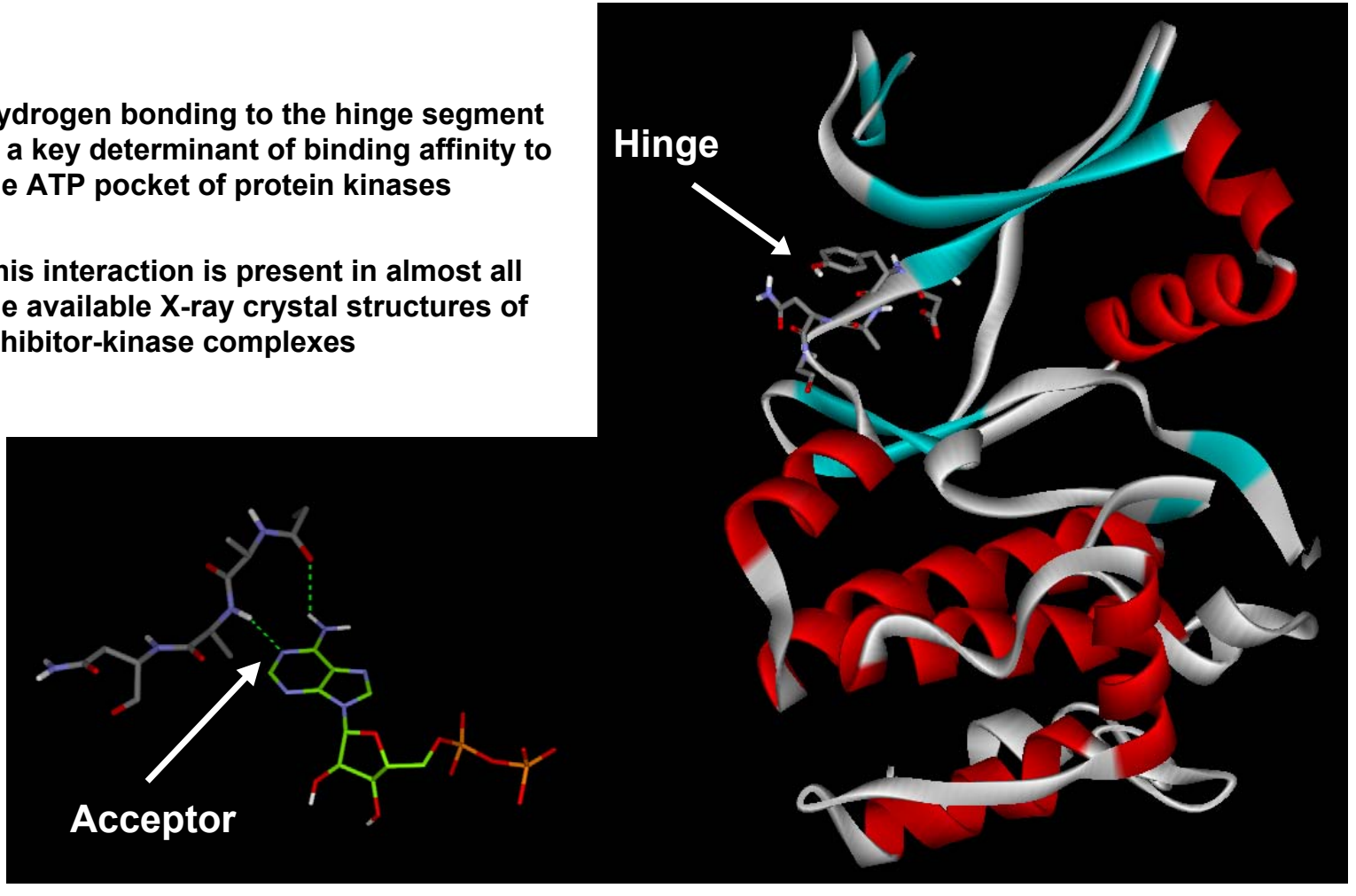
Example of knowledge : Protein kinase family

Hydrogen bonding to the hinge segment is a key determinant of binding affinity to the ATP pocket of protein kinases

This interaction is present in almost all the available X-ray crystal structures of inhibitor-kinase complexes



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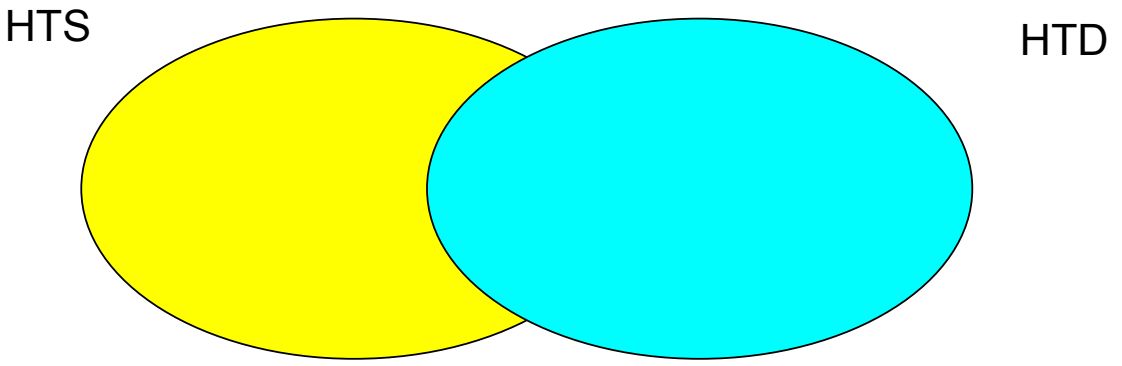
HTS *vs* HTD hits

These two approaches are complementary



- **Strong binders are generally found**
- **Compounds consuming**
- **Expensive**
- **Assay format suitable for HTS**
- **Low rate of false negatives**

- **Possibility to have access to weak binders**
- **Low cost method**
- **Easy to set up**
- **3D structure of the target needed**
- **High rate of false negatives/positives**

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Application of High-Throughput Docking

- **HTS experiment unavailable**
  - **Difficult or impossible to set up**
  - **Explanatory project**
- **In addition to HTS**
  - **Follow-up of HTS (virtual screen of recently added libraries)**
- **In parallel with HTS**
  - **Enrich HTS hit list with hits found *in silico***
- **Virtual screening of compounds not available in-house**
  - **Commercial compounds**
  - **Virtual compounds (easily accessible, e.g. by combinatorial chemistry)**

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CK2 - Target

Protein kinase CK2 (casein kinase II)

- **Potential therapeutic target in oncology**
- **Potent and selective small molecule inhibitor required**

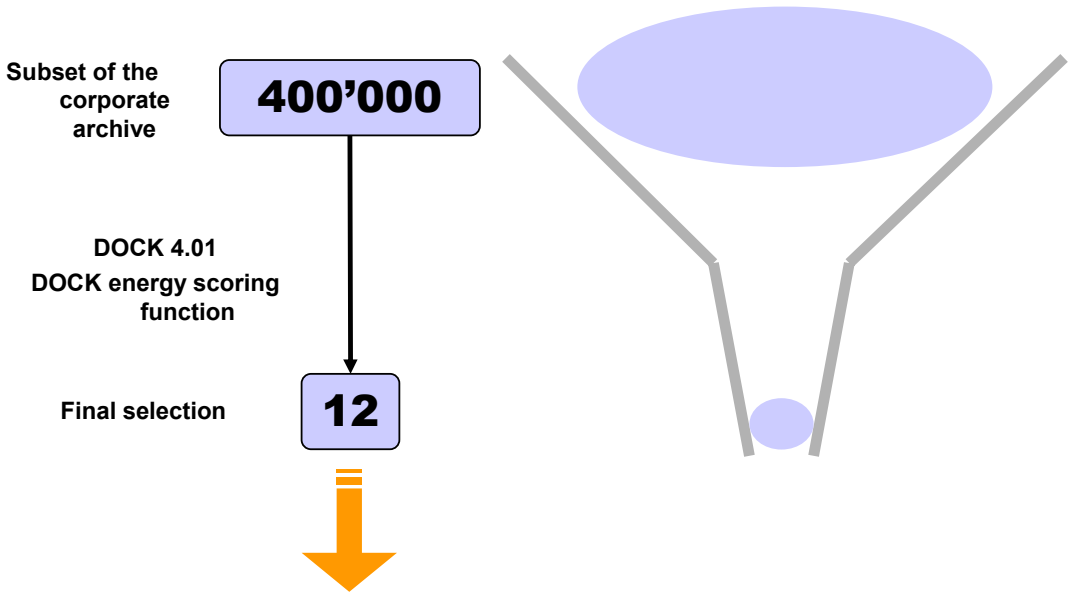
- **X-ray unavailable**
- **Homology model of Human CK2 possible**
  - **72% sequence identity with Zea Mays CK2**
  - **82% sequence identity considering the ATP binding site**

```
Zea mays   34   YEVVRKVGRGKYSEVFEGINVNNNEKCIIKILKPVKKKKIKREIKILQNLCGGPNIVKLLDIVRDQHSKTPSLIFEYVNNTDFKVLYPTLTDYDIRYYIY
                 xx   x          x  xx    Xxx                 x  X     xX X   x XX x  x x  x       X  X        x x
Human      39   YQLVRKLGRGKYSEVFEAINITNNEKVVVKILKPVKKKKIKREIKILENLRGGPNIITLADIVKDPVSRTPALVFEHVNNTDFKQLYQTLTDYDIRFYMY

Zea mays  134   ELLKALDYCHSQGIMHRDVKPHNVMIDHELRKLRLIDWGLAEFYHPGKEYNVRVASRYFKGPELLVDLQDYDYSLDMWSLGCMFAGMIFRKEPFFYGHDN
                 x         x                 X               x                   X X            x x         x
Human     139   EILKALDYCHSMGIMHRDVKPHNVMIDHEHRKLRLIDWGLAEFYHPGQEYNVRVASRYFKGPELLVDYQMYDYSLDMWSLGCMLASMIFRKEPFFHGHDN

Zea mays  234   HDQLVKIAKVLGTDGLNVYLNKYRIELDPQLEALVGRHSRKPWLKFMNADNQHLVSPEAIDFLDKLLRYDHQERLTALEAMTHPYF
                x    x       xX XX xx  X     xxxXxx      X Xx xxxx         x            X    X   X
Human     239   YDQLVRIAKVLGTEDLYDYIDKYNIELDPRFNDILGRHSRKRWERFVHSENQHLVSPEALDFLDKLLRYDHQSRLTAREAMEHPYF
```

CK2 - HTD Experiment



CK2 phosphorylation inhibition assay

- **4 compounds have % inhibition value > 50 @10µM**
- **Belong to different chemical classes**
- **Most potent has an IC_{50} value of 80nM and was most potent inhibitor of the CK2 kinase ever reported (J. Med. Chem. 46, 2656–2662)**

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Follow-up of HTS

Backup lead compound needed in a medicinal project

- **HTS performed several years ago**
- **X-ray structure not available but homology model possible**
  - **HTD allows to virtually screen compounds not already screen by HTS**

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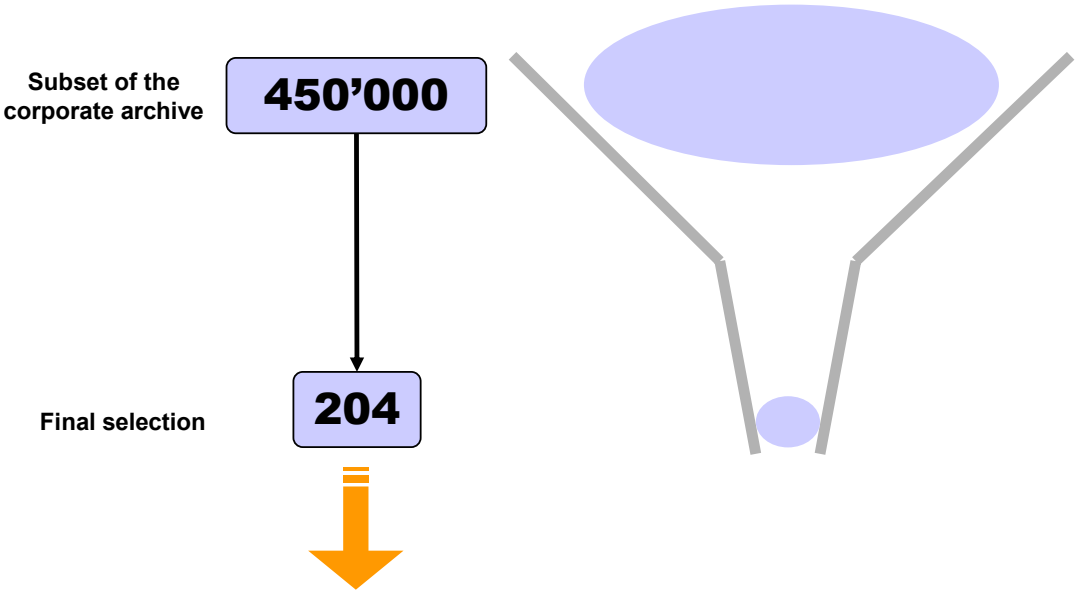
Follow-up of HTS



Biological assay

- 33 compounds found to be active
- 16 different chemical classes
- follow-up chemistry initiated

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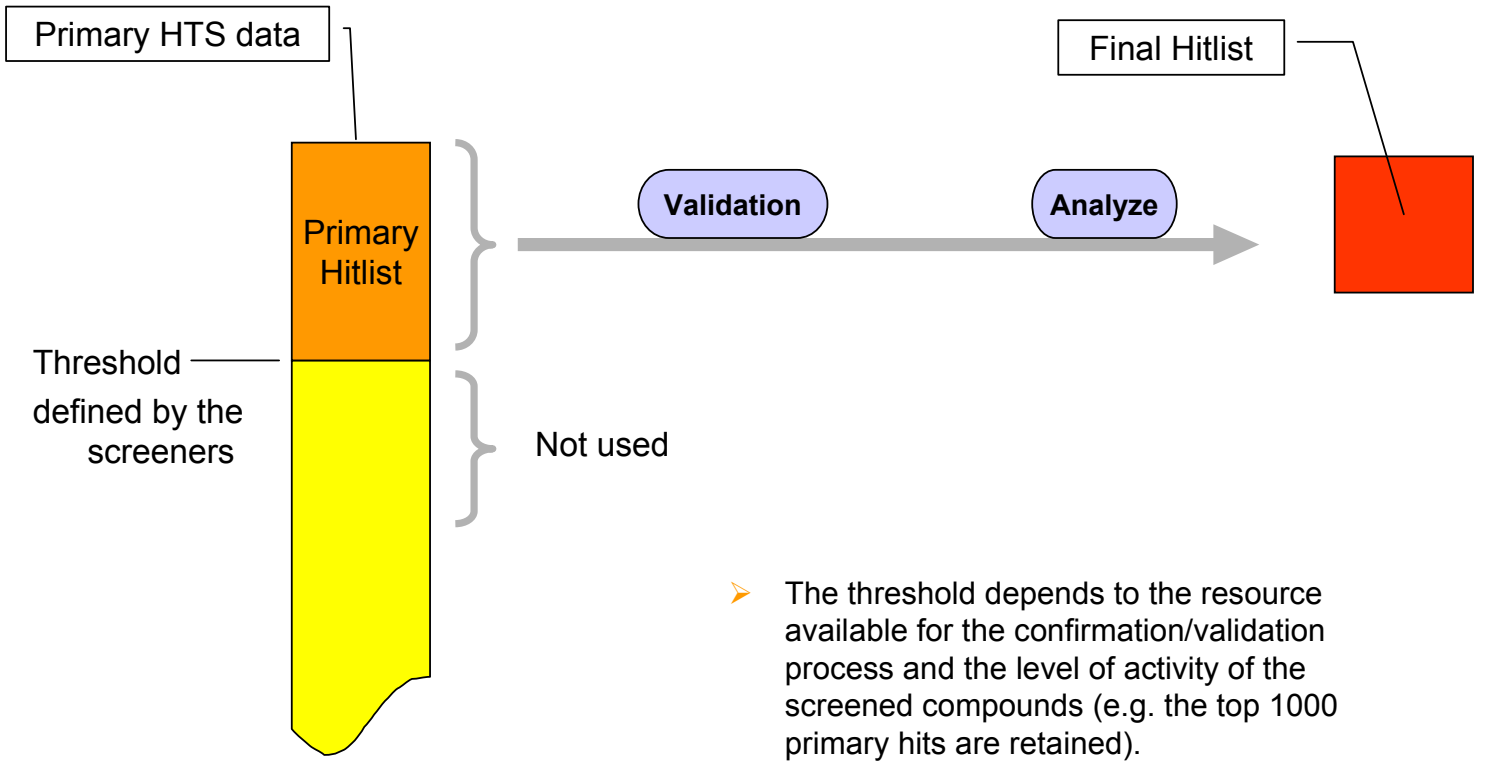
HTS Process



- The threshold depends to the resource available for the confirmation/validation process and the level of activity of the screened compounds (e.g. the top 1000 primary hits are retained).

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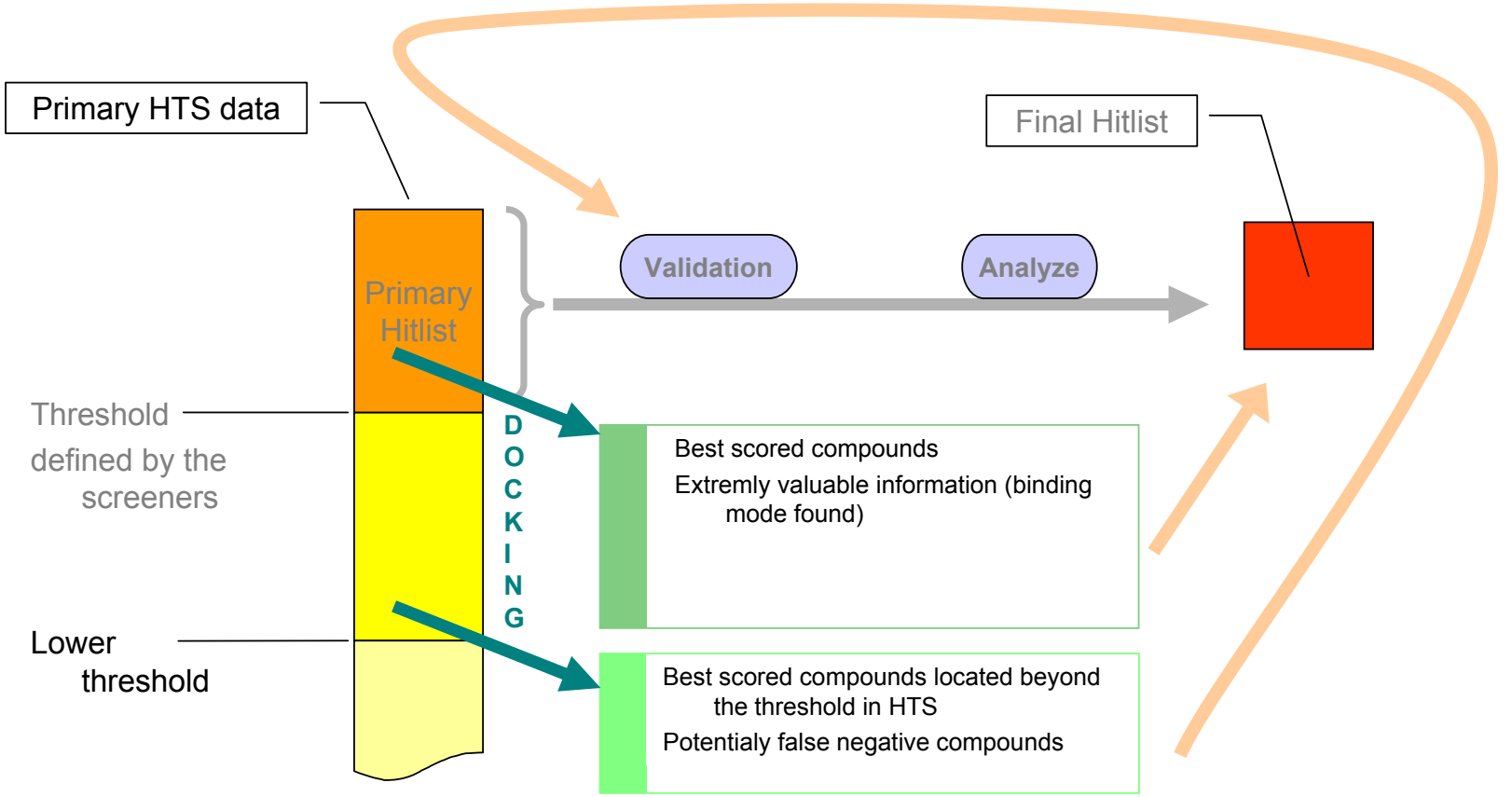
Combined HTS and HTD Process



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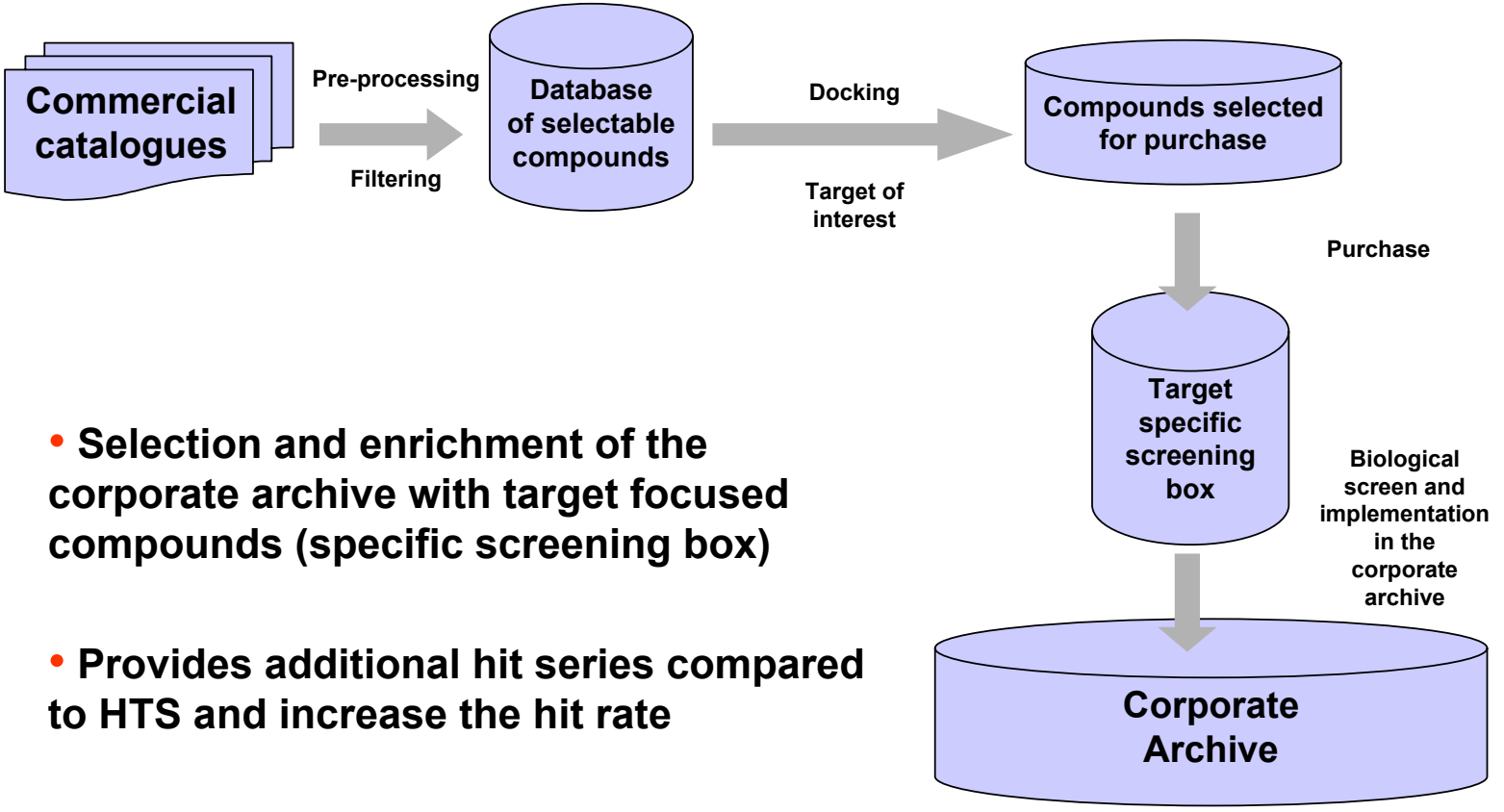
Commercial compounds screening



- **Selection and enrichment of the corporate archive with target focused compounds (specific screening box)**
- **Provides additional hit series compared to HTS and increase the hit rate**

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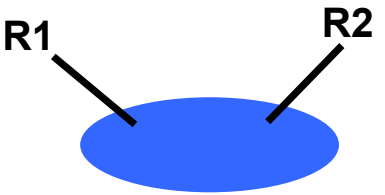
Virtual compounds screening

- **Structure assisted optimization of leads**
- **Targeted Combinatorial Chemistry**
- **Evaluation of synthetically accessible libraries around a given lead**
  - Identify feasible synthetic routes
  - Assemble list of relevant available reagents
  - Build virtual library by attaching reagents to scaffold
  - Evaluate virtual library by docking into target binding site
- **Can be done iteratively**



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Virtual library Screening

Need to improve affinity and ADME profile of a lead in a medicinal project

- **A part of the structure is conserved**

- **Virtual library created by coupling the conserved part and all the reagents easily available**

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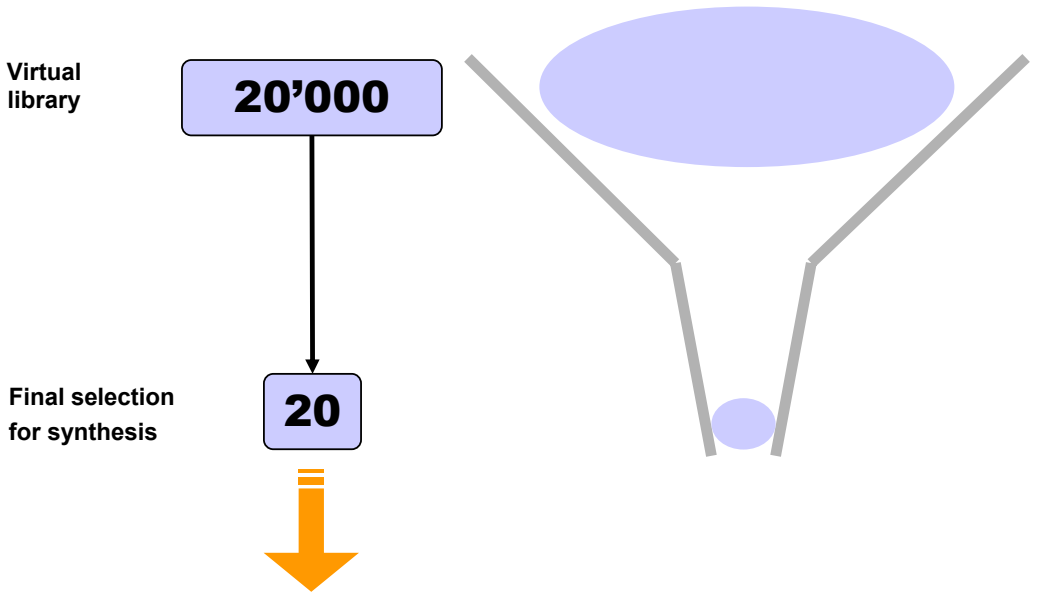
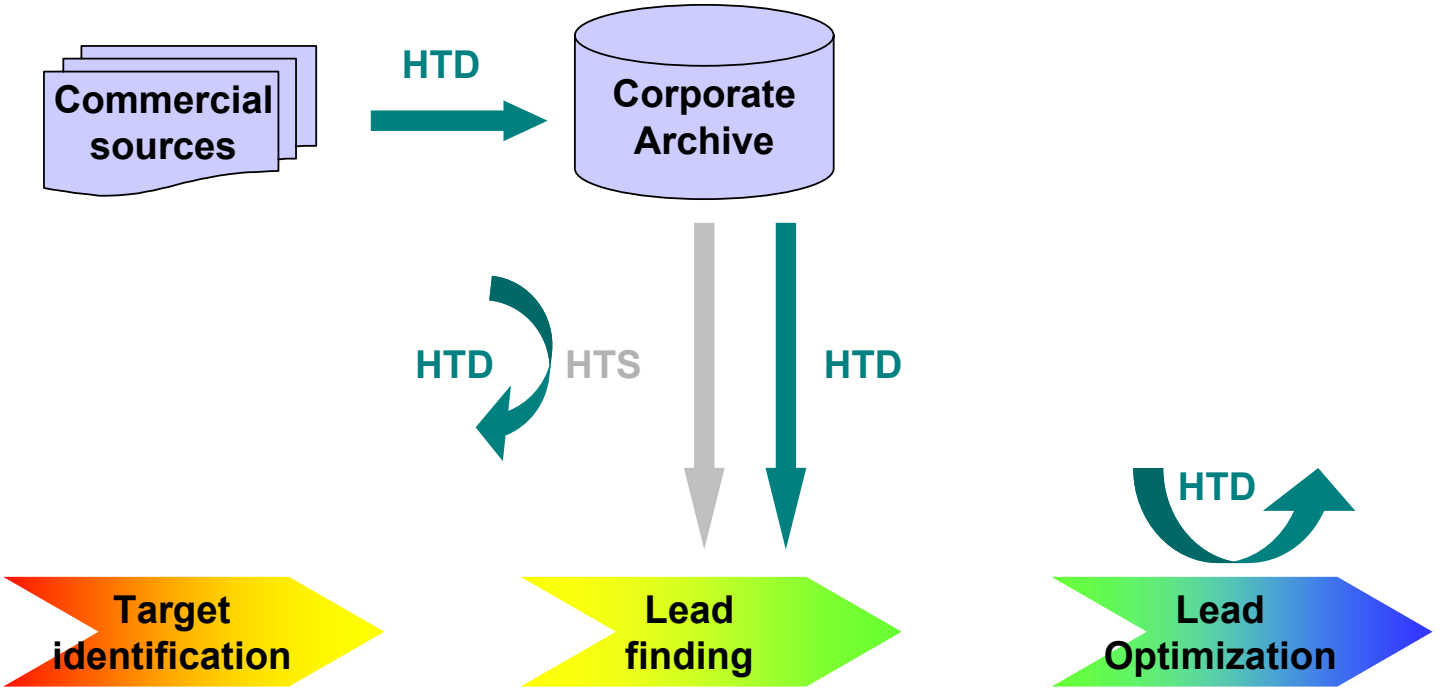
Virtual library Screening



Synthesis / Biological assay

- **4 compounds found to be active**
- **1 has contributed to the discovery of a new lead compound with improved affinity and ADME profile**

Summary



High-Throughput Docking can make significant contributions to the lead discovery process.

Acknowledgements

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