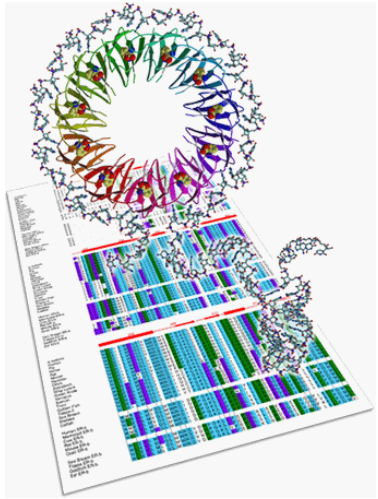
Escuela Complutense de Verano
Especialista en Bioinformática



Interacciones entre proteínas y pequeños ligandos (I)

Federico Gago
Departamento de Farmacología
Universidad de Alcalá, Madrid

Interacciones entre proteínas y moléculas pequeñas

1. Concepto de ligando y sitio de unión. Ejemplos.
2. Bases de datos estructurales y programas asociados.
3. Caracterización estructural de moléculas pequeñas y sus complejos con proteínas.
4. Acoplamiento ligando-receptor ("docking"): algoritmos y programas.
5. Cribado virtual.

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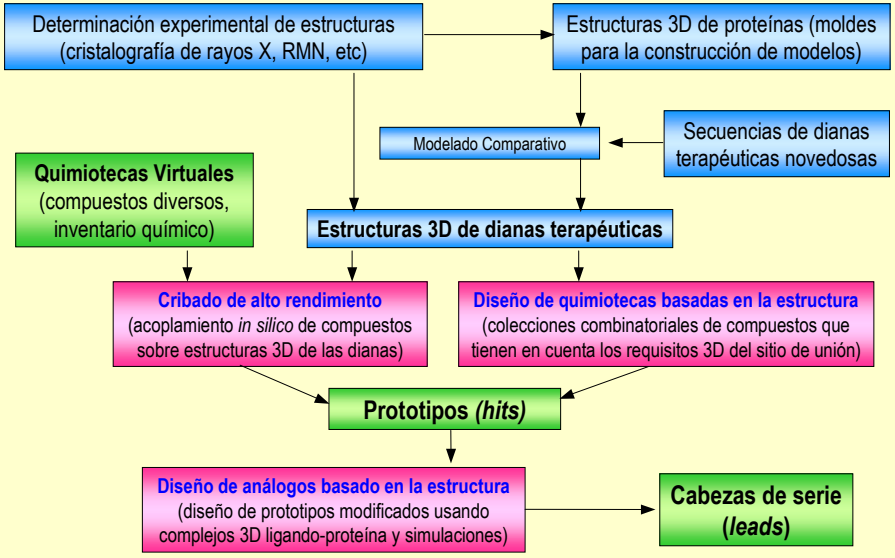
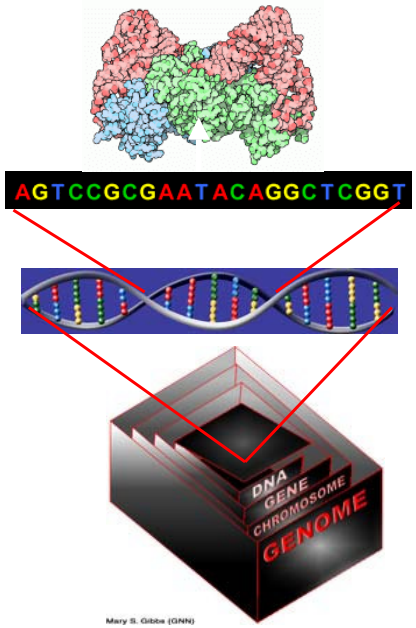
6. Relaciones estructura-actividad: QSAR y 3D-QSAR.
7. Diseño de nuevos ligandos.

Integración de Metodologías Informáticas y Basadas en la Estructura para el Descubrimiento de Fármacos



Gene expression = Protein production



How Proteins Work

Proteins recognize and reversibly bind to other molecules: *cofactors, substrates, inhibitors*… Also *ions* and other *proteins*.

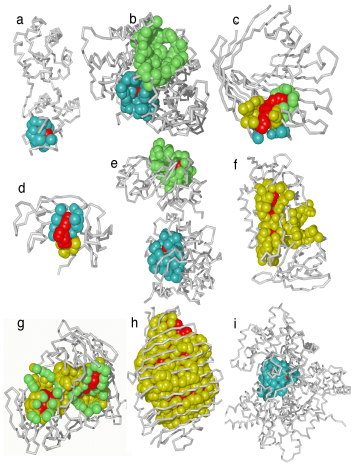
The bound small molecule is called a **ligand**.

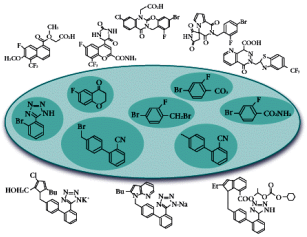
In the case of enzymes, the region that associates with substrates and products is called the **active site**.

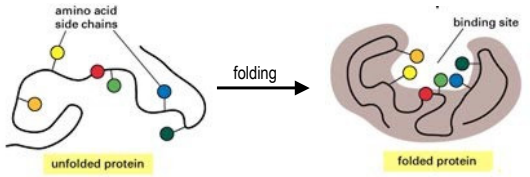
The region of a protein that associates with activator or inhibitor molecules is called an **allosteric site**.

Proteins can have > 1 binding site for different ligands

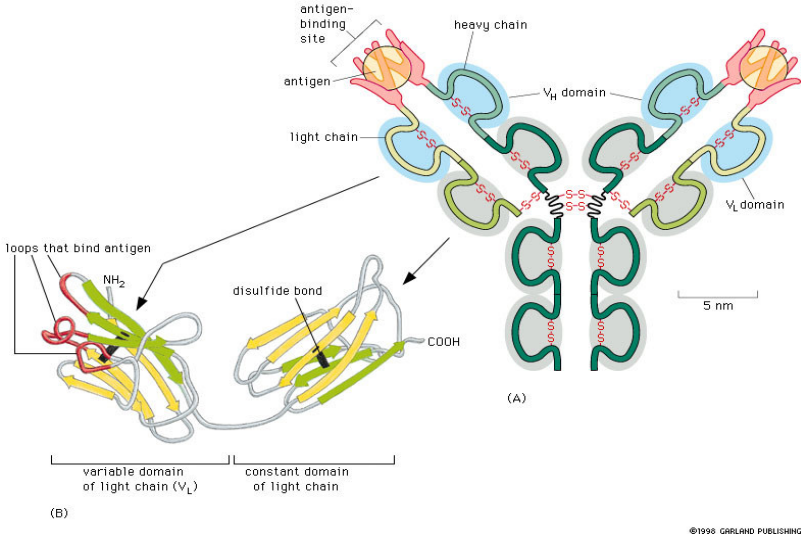
Proteins fold in such a way that they create specific sites that are the right *size*, *shape*, and *polarity* for their ligands.



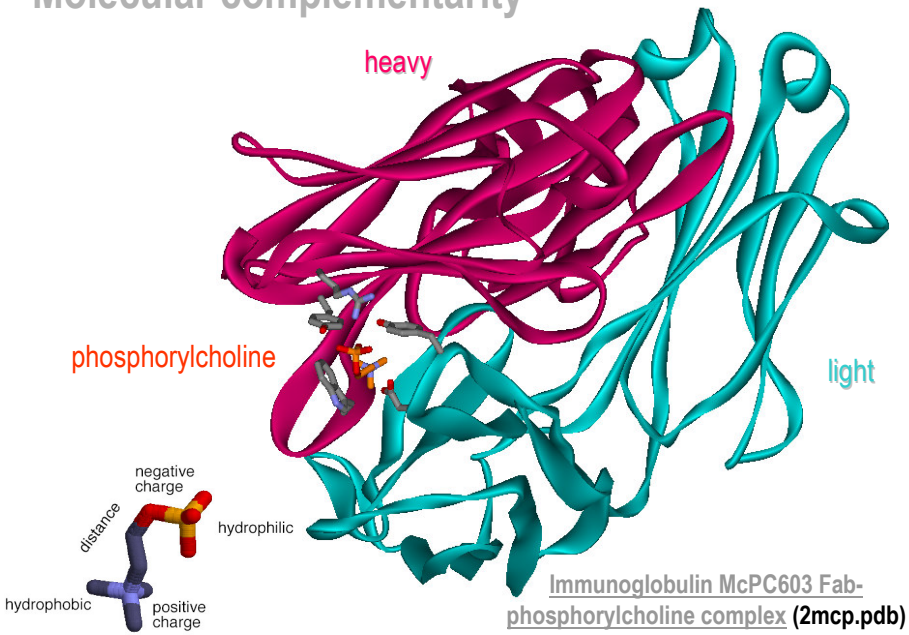




Antibodies selectively bind to antigens

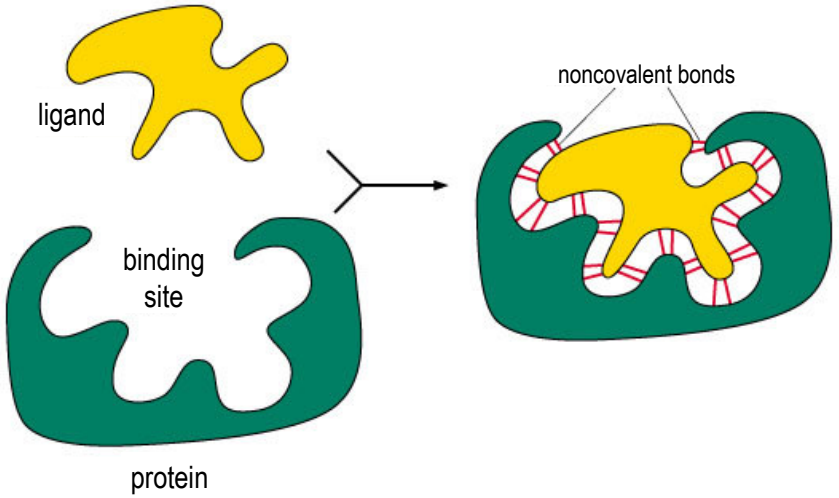


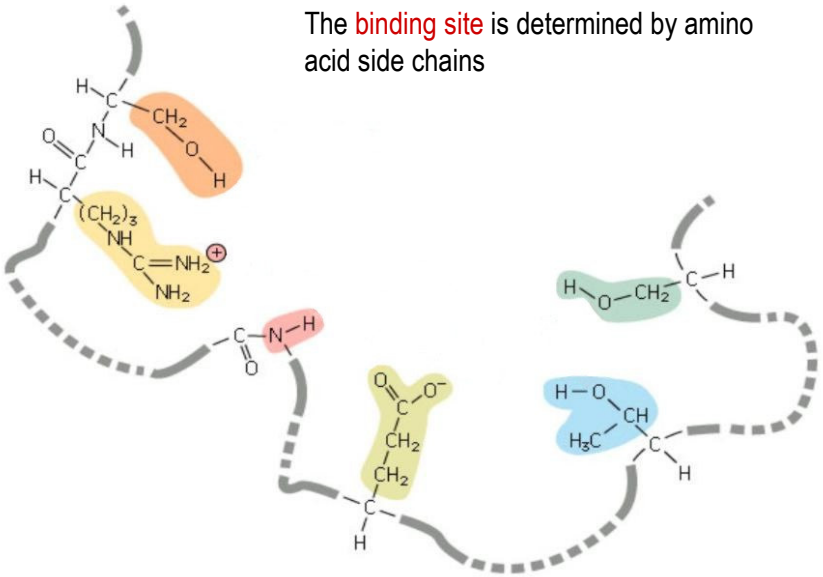
Molecular complementarity



Immunoglobulin McPC603 Fab-phosphorylcholine complex (2mcp.pdb)

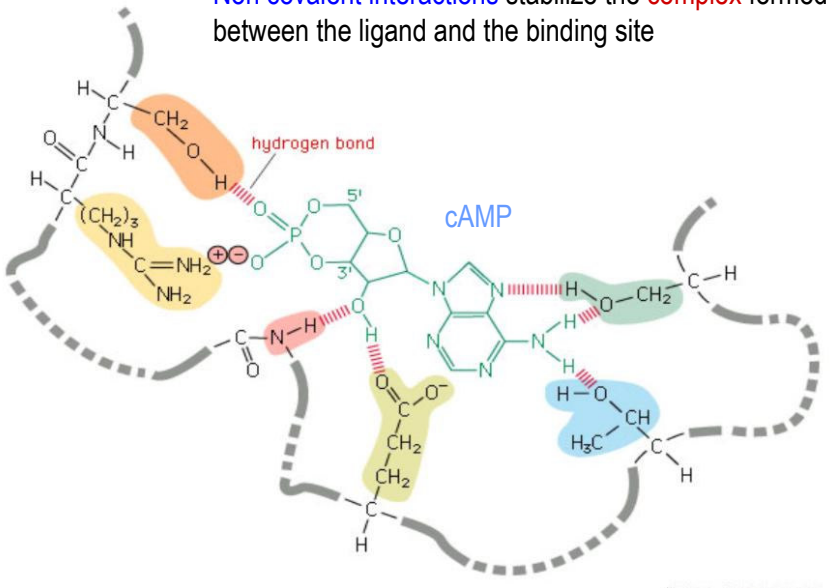
Ligand binding is highly selective



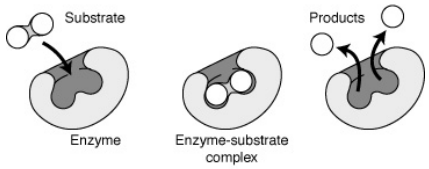


The binding site is determined by amino acid side chains

Non covalent interactions stabilize the complex formed between the ligand and the binding site



Enzymes: A special case of protein function

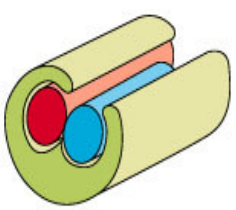


Enzymes bind and assist in the chemical transformation of other molecules

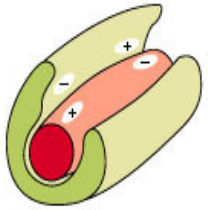
Substrate: molecule acted upon by an enzyme (analogous to ligand)

Catalytic site: substrate-binding site (analogous to ligand-binding site)

How do enzymes catalyze reactions?



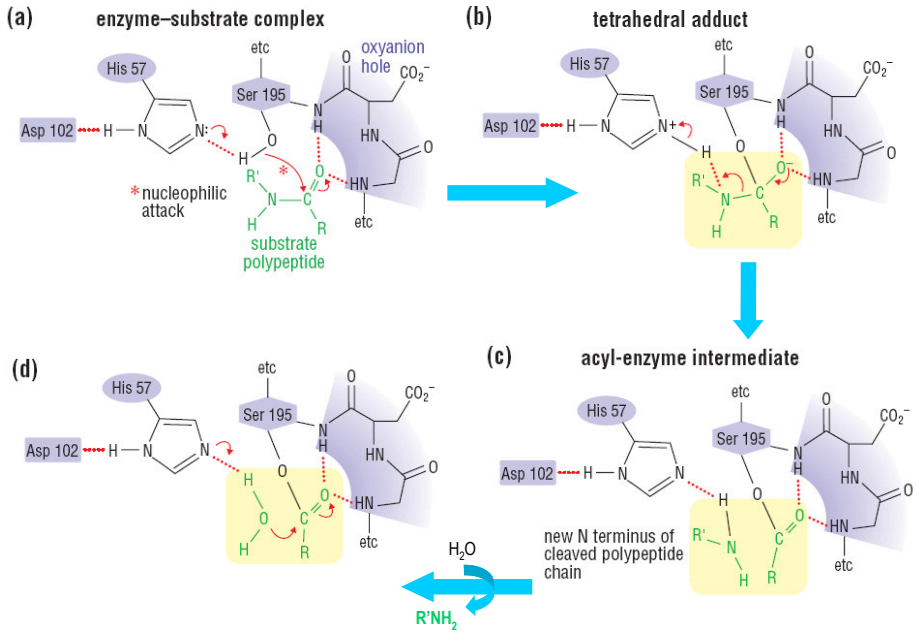
(A) enzyme binds to two substrate molecules and orients them precisely to encourage a reaction to occur between them



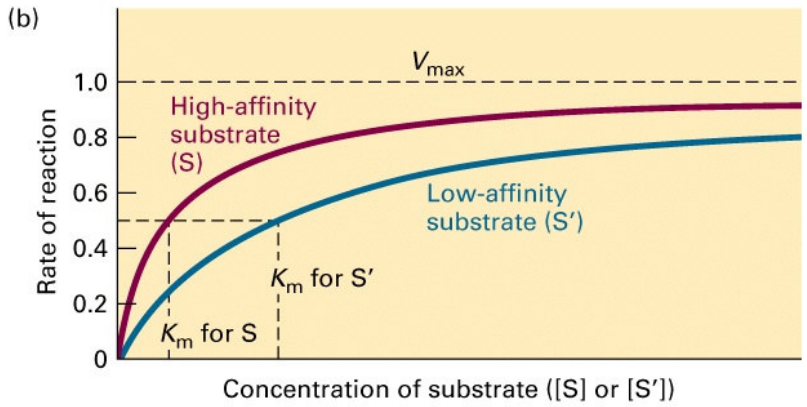
(B) binding of substrate to enzyme rearranges electrons in the substrate, creating partial negative and positive charges that favor a reaction

(C) enzyme strains the bound substrate molecule, forcing it toward a transition state to favor a reaction

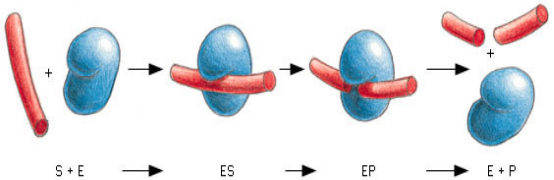
The chemical steps in peptide hydrolysis catalyzed by the serine protease chymotrypsin



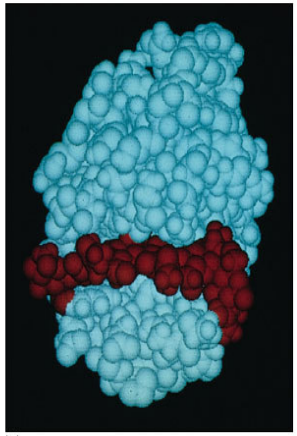
Kinetics of an enzymatic reaction are described by V_{max} and K_m



Lysozyme catalyzes the cutting of a polysaccharide chain

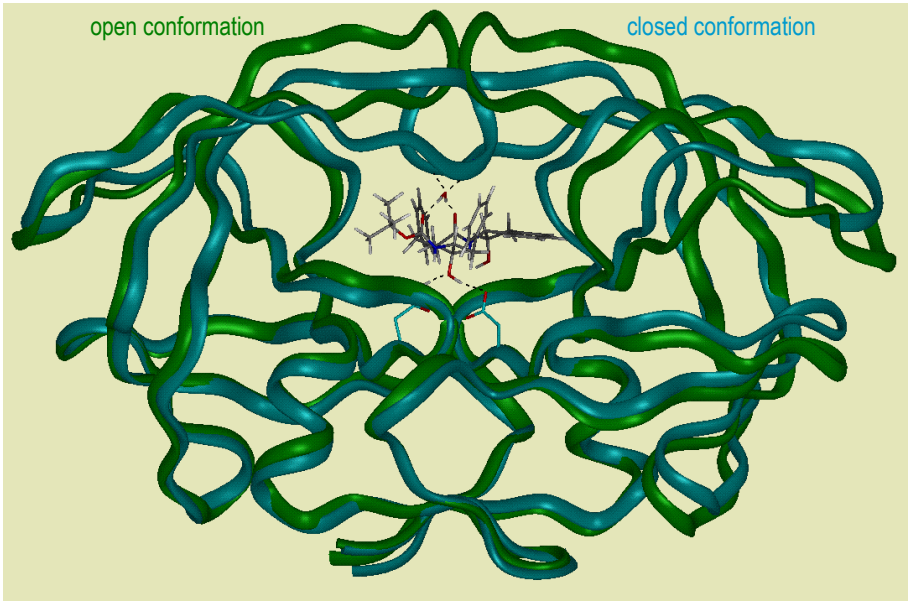


(A)

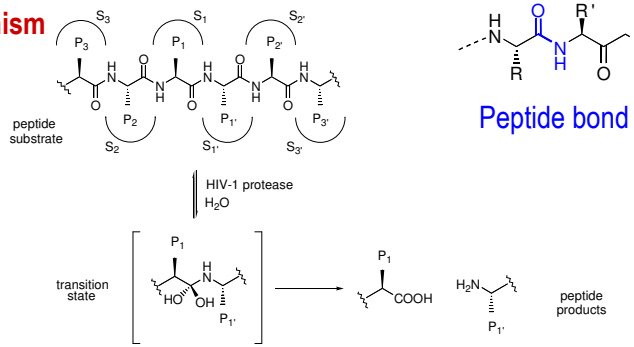


(B)

HIV-1 protease

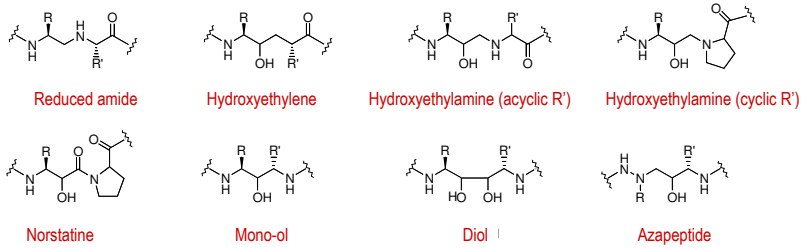


Hydrolytic mechanism in HIV-1 protease



Peptide bond

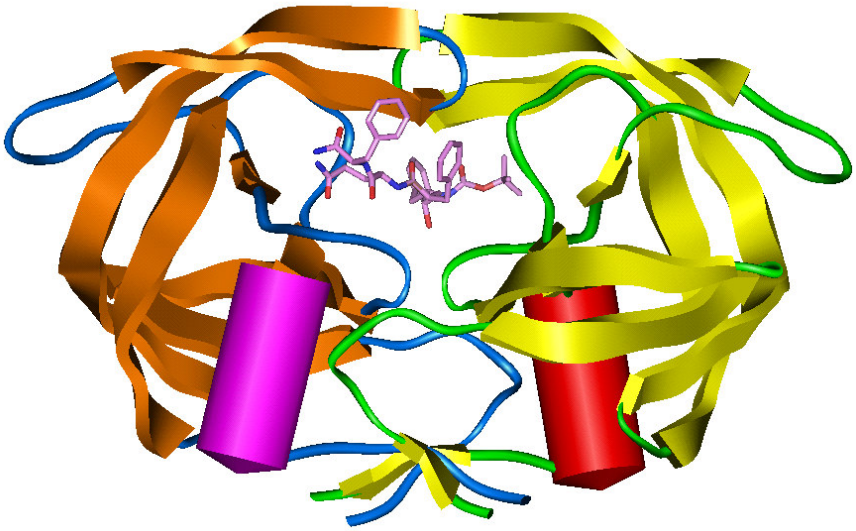
Examples of non-hydrolyzable isosteres of the peptide bond cleaved by HIV-1 protease



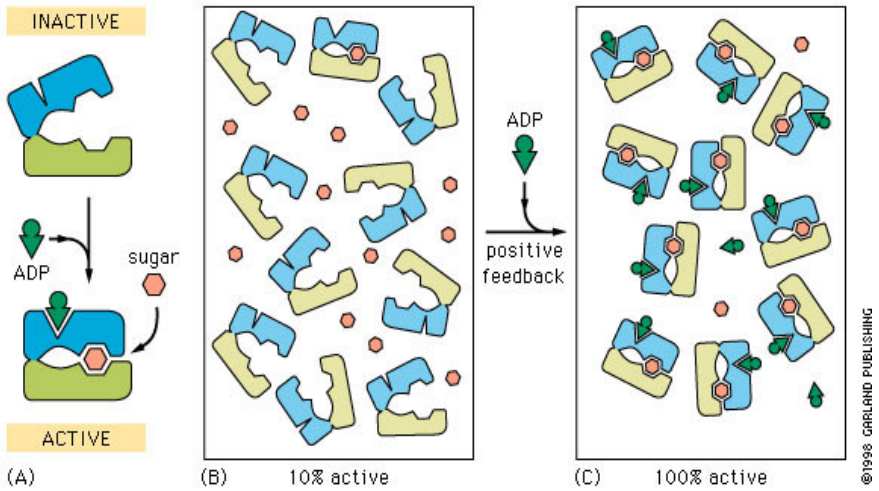
Reduced amide | Hydroxyethylene | Hydroxyethylamine (acyclic R') | Hydroxyethylamine (cyclic R')

Norstatine | Mono-ol | Diol | Azapeptide

HIV-1 protease in complex with inhibitor QF-34

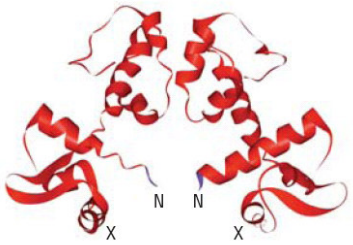


Enzyme activation caused by an allosteric change

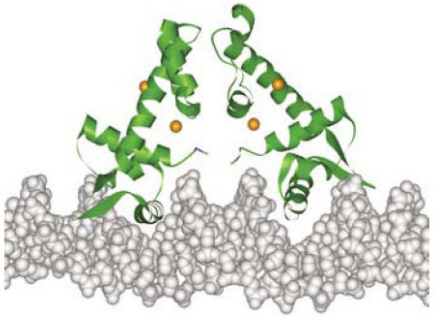


Binding of gene regulatory proteins to DNA is often controlled by ligand-induced conformational changes

Iron binding regulates the repressor of the diphtheria toxin gene:

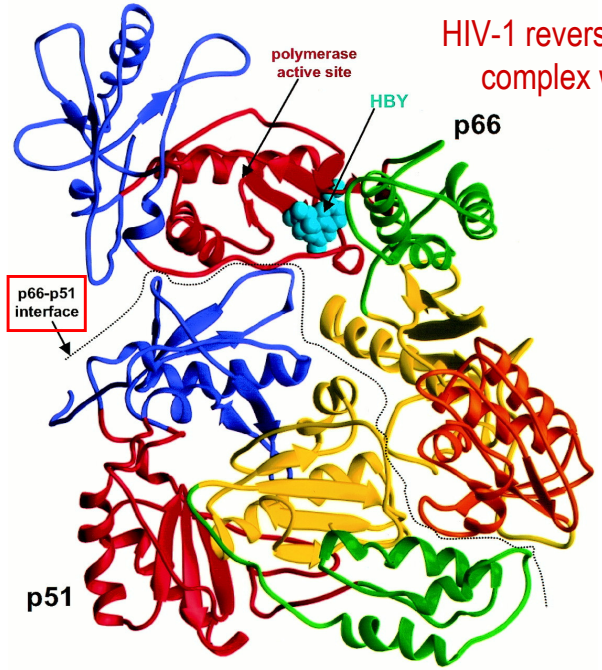


aporepressor DtxR (PDB 1dpr)



ternary complex of **repressor** (green), metal ion (Fe^{2+}) and DNA (PDB 1fst).

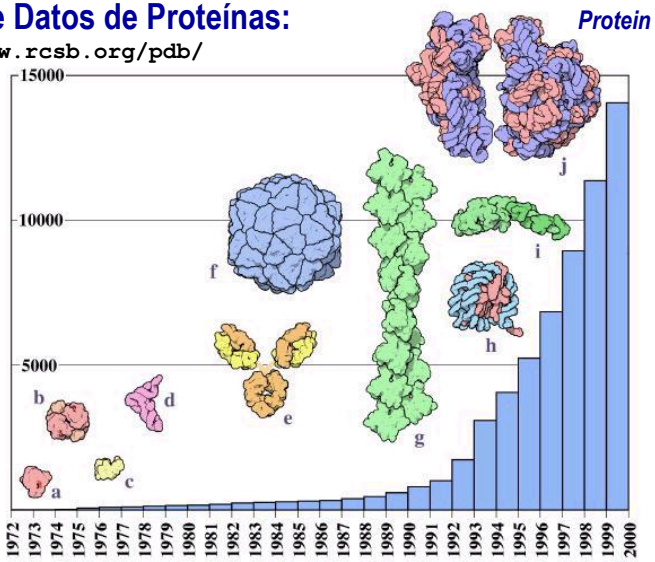
HIV-1 reverse transcriptase in complex with inhibitor HBY



Banco de Datos de Proteínas:

Protein Data Bank

http://www.rcsb.org/pdb/



(a) mioglobina (b) hemoglobina (c) lisozima (d) ARN de transferencia
(e) anticuerpos (f) virus (g) actina (h) el nucleosoma
(i) miosina (j) el ribosoma

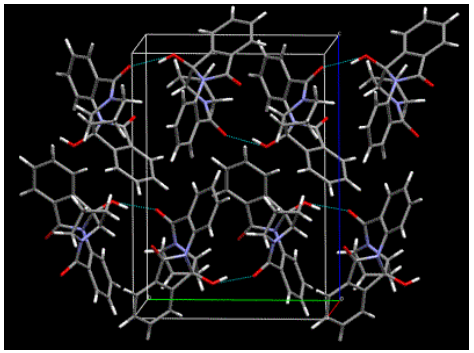
Cortesía de David Goodsell, TSRI

Cambridge Structural Database

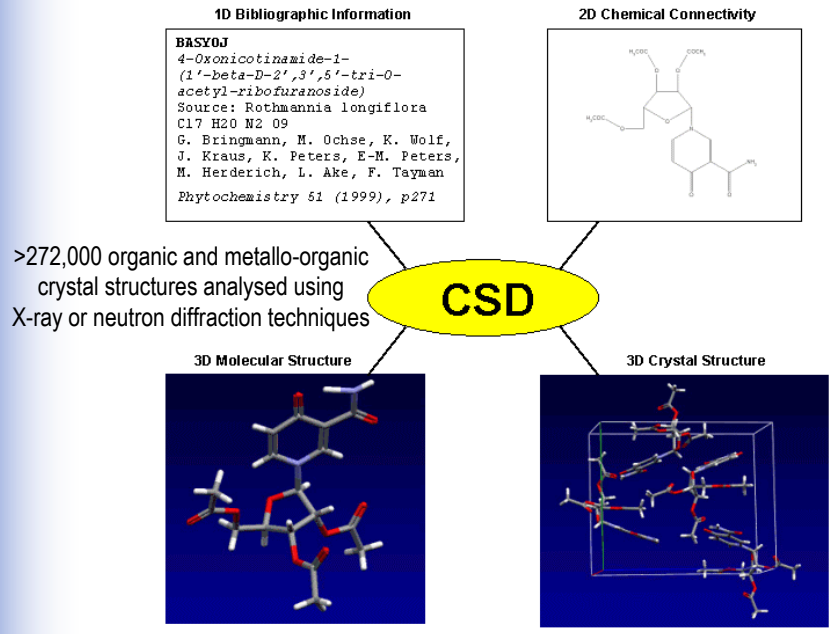
The Cambridge Crystallographic Data Centre (CCDC) builds, maintains and distributes the Cambridge Structural Database (CSD), a searchable database of organic and metallo-organic crystal structures.

The CCDC also produce and distribute software products which make use of the data contained in the CSD.

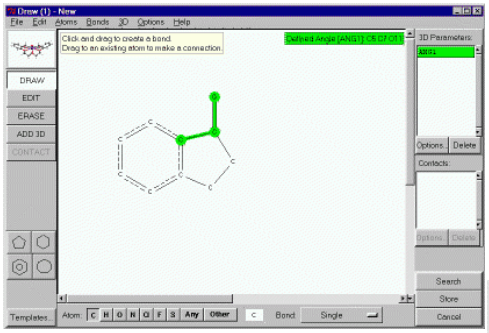
Increasing the Value of Crystallographic Databases



- Derived knowledge bases
- Knowledge-based applications programs
- Data mining tools for protein-ligand complexes

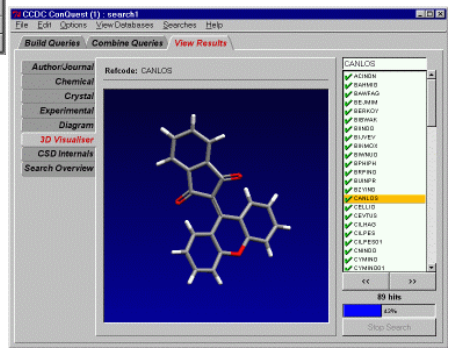


ConQuest



ConQuest provides a full range of text/numeric database search options, in addition to more complex search functionality, including:

- **Chemical substructure searching**
- **Geometrical searching**
- **Intermolecular non-bonded contact searching**



Cambridge Crystallographic Data Centre

http://www.ccdc.cam.ac.uk/

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COMPND    NICOTINE MONOHYDROGEN IODIDE
CRYST    13.705    9.974    8.436  90.00  90.00  90.00 P212121
ATOM      1  I1  NICO    1        1.583   1.561   0.355  1.00  0.00
ATOM      2  N1  NICO    1        5.355  -5.377  10.252  1.00  0.00
ATOM      3  C1  NICO    1        5.744  -4.111  10.057  1.00  0.00
ATOM      4  C2  NICO    1        5.375  -3.343   8.953  1.00  0.00
ATOM      5  C3  NICO    1        4.573  -3.927   7.982  1.00  0.00
ATOM      6  C4  NICO    1        4.180  -5.249   8.151  1.00  0.00
ATOM      7  C5  NICO    1        4.584  -5.922   9.302  1.00  0.00
ATOM      8  C6  NICO    1        5.840  -1.905   8.899  1.00  0.00
ATOM      9  C7  NICO    1        4.752  -0.841   8.748  1.00  0.00
ATOM     10  C8  NICO    1        5.461   0.374   8.127  1.00  0.00
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ATOM     12  N2  NICO    1        6.722  -1.627   7.699  1.00  0.00
....
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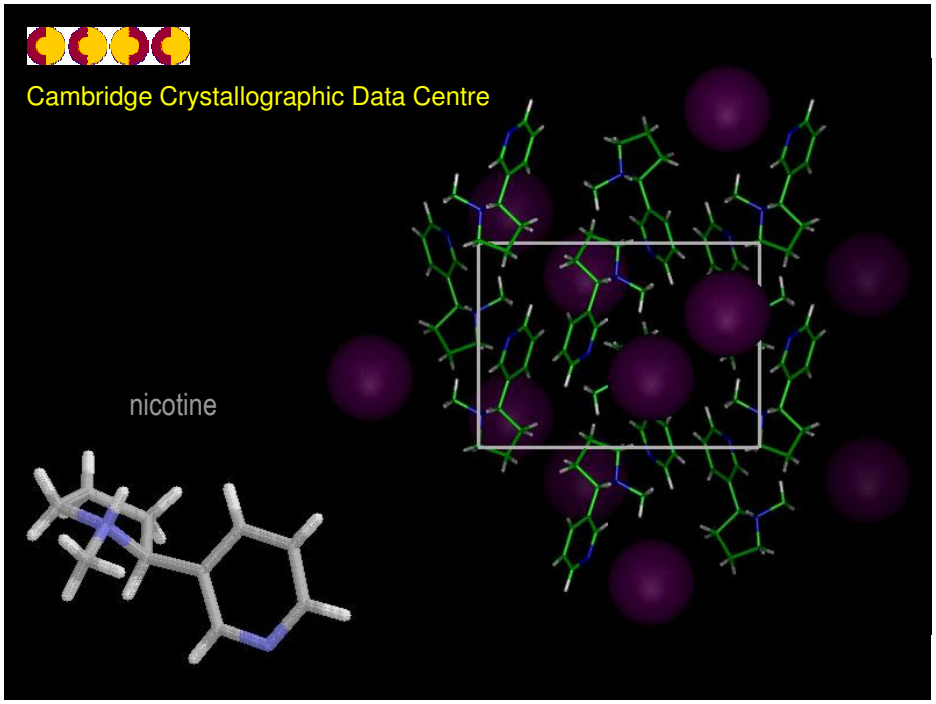
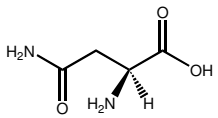
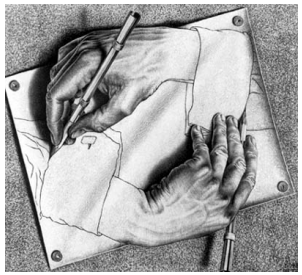


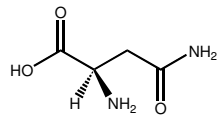
The Importance of Chirality



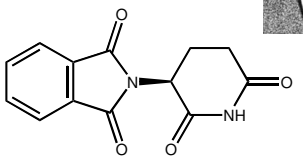
Bitter

Asparagine



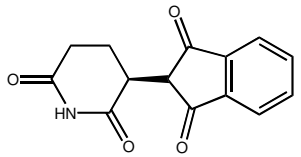


Sweet



Extreme teratogen

Thalidomide

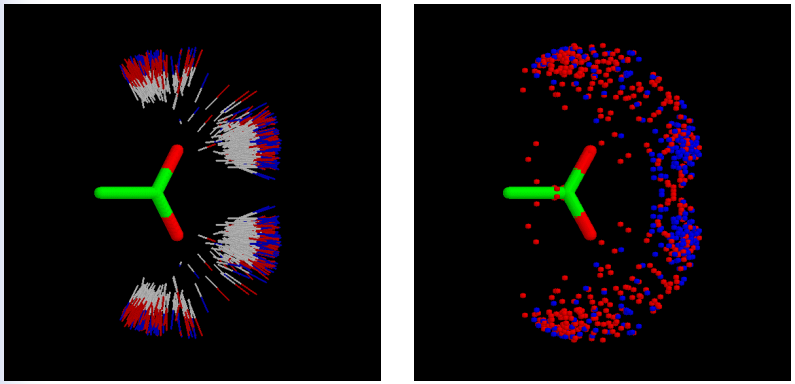


Anti-morning sickness

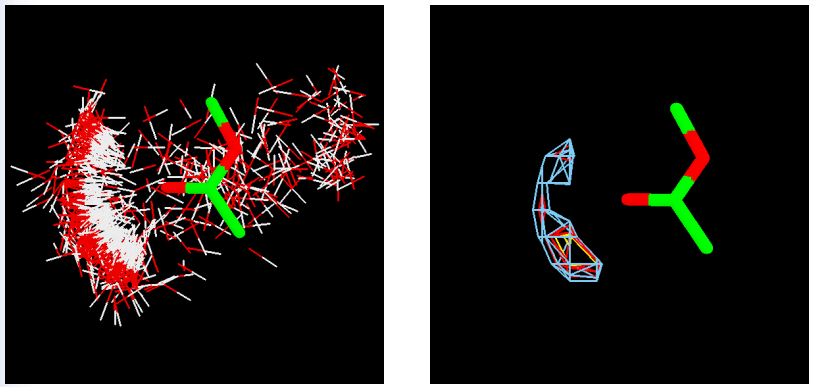
IsoStar and SUPERSTAR

- IsoStar - knowledge base of information about intermolecular interactions
- SuperStar - program for predicting binding points in an enzyme active site
- SuperStar predictions based solely on IsoStar data

IsoStar Scatterplots



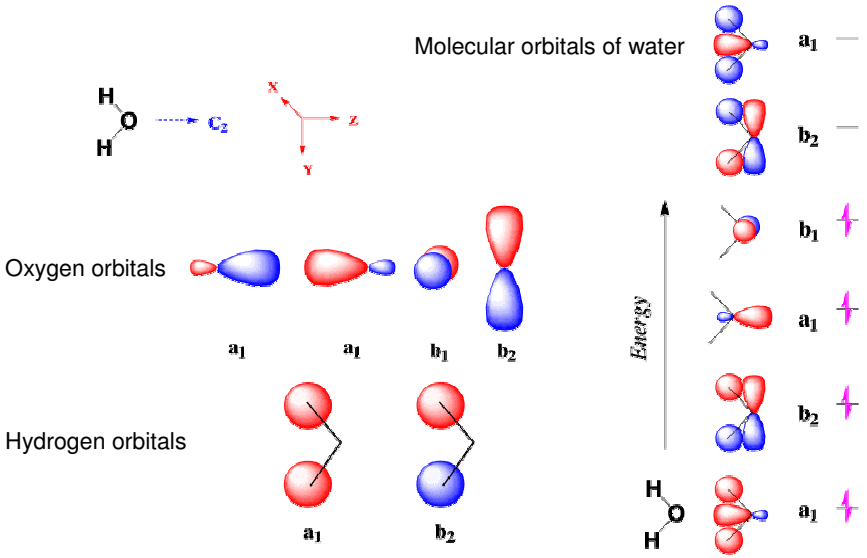
IsoStar Density Surfaces



A probability surface derived from the observed positions of hydrogen-bonding hydrogen atoms around aliphatic esters.

Quantum Chemistry

Atomic orbitals can be combined to give molecular orbitals



Ab initio METHODS

* Hartree-Fock method
* Electron correlation methods
  - variational methods

    Configuration Interaction with double excitations (CID)

    Configuration Interaction with single and double excitations (CISD)
  - perturbation methods

    Møller and Plesset (MP2, MP3, MP4)

    Quadratic Convergence CI method (QCISD)
  - density functional methods (DFT)

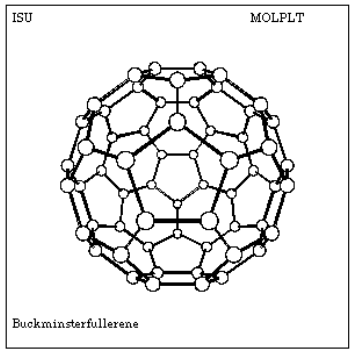
    BP86 - developed by Becke and Perdew in 1986

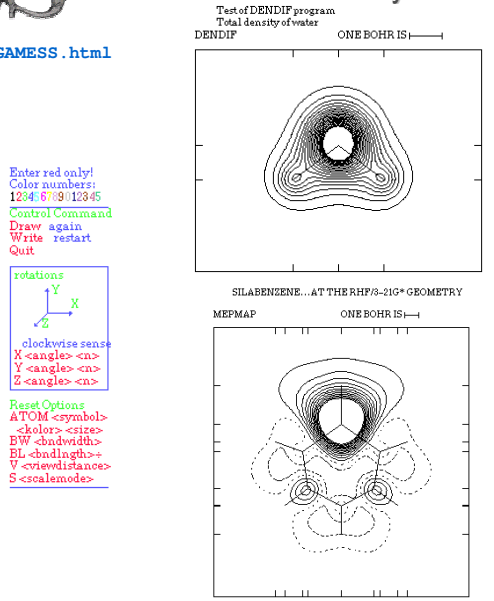
    BLYP - developed by Becke, Lee, Yang and Parr

    B3LYP - a modification of BLYP in which a 3-parameter functional developed by Axel Becke is used.

GAMESS General Atomic and Molecular Electronic Structure System

http://www.msg.ameslab.gov/GAMESS/GAMESS.html





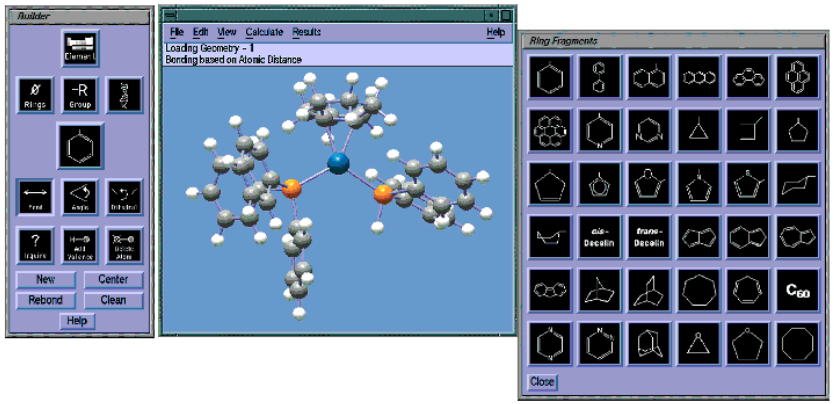
Gaussian

http://www.gaussian.com/

wavefun.com
chemistry software tools
for education and industry

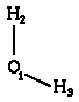
Spartan

http://www.wavefun.com/



Some sample Gaussian z-matrices

Water (C_{2v})



With variables:

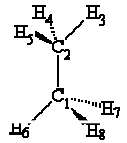
```
o
h 1 11
h 1 11 2 a1

11 0.96
a1 104.0
```

With values:

```
o
h 1 0.96
h 1 0.96 2 104.0
```

Ethane (D_{3d})




```
c
c 1 11
h 2 12 1 a1
h 2 12 1 a1 3  120.0
h 2 12 1 a1 3 -120.0
h 1 12 2 a1 3  180.0
h 1 12 2 a1 6  120.0
h 1 12 2 a1 6 -120.0

11 1.54
12 1.09
a1 110.0
```

SEMI-EMPIRICAL METHODS: levels of approximation

CNDO Complete Neglect of Differential Overlap (Developed by John Pople - assumes atomic orbitals to be spherical when evaluating the two-electron integrals)

INDO Intermediate Neglect of Differential Overlap

NDDO Neglect of Diatomic Differential Overlap

MINDO/3 Modified INDO (Developed by Michael Dewar - uses a set of parameters to approximate the two-electron repulsion integrals)

ZINDO Includes parameters for transition metals

MNDO Modified NDO (Developed by Michael Dewar and Walter Thiel in 1977)

AM1 Austin Model 1 (Developed by Michael Dewar and Andrew Holder in 1986)

PM3 Parametric Model 3 (Developed by Jimmy Stewart in 1988)

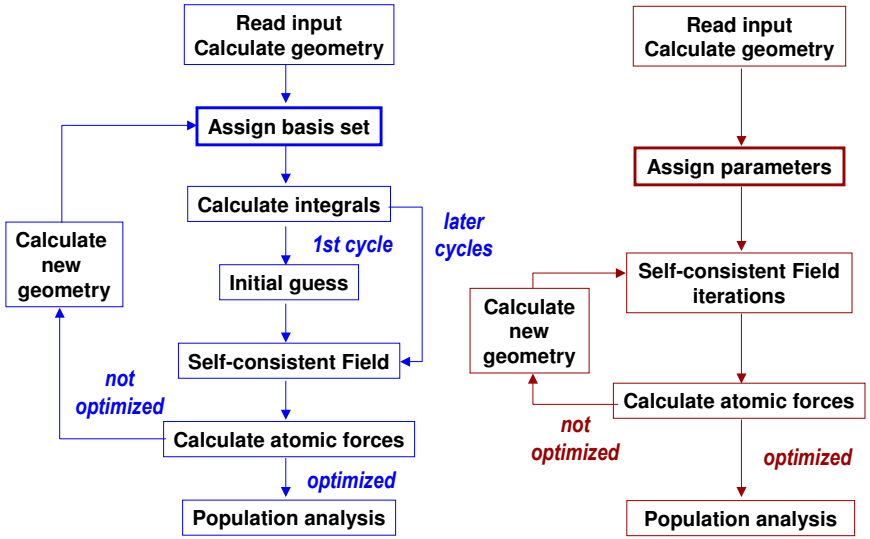
Sample input for MOPAC

```
PM3 EF PRECISE
H2O (water)
MOPAC input as a Z-matrix
O
H          0.96000  1                                    1
H          0.96000  1     104.00000  1                   1 2
```

Water (C_{2v})


```
AM1 EF PRECISE
H2O (water)
MOPAC input in Cartesian coordinates
O         0.0000  0  0.0000  0  0.0000  0
H         0.9600  1  0.0000  0  0.0000  0
H        -0.2322  1  0.9315  1  0.0000  0
```

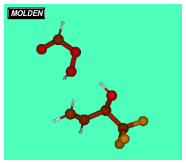
Typical flow charts for an *ab initio* optimization and a corresponding semi-empirical calculation



MOLDEN http://www.cmbi.kun.nl/~schaft/molden/molden.html

a pre- and post processing program of molecular and electronic structure

- ✓ reads all the required information from the GAMESS / GAUSSIAN / MOPAC outputfiles, and is also capable of importing lots of other formats (ChemX, PDB, etc)
- ✓ displays Molecular Density and Molecular Orbitals
- ✓ supports contour plots, 3-d grid plots with hidden lines and a combination of both.
- ✓ can write a variety of graphics instructions; postscript, XWindows, VRML, povray, OpenGL, tekronix4014, hpgl, hp2392 and Figure.



- ✓ can animate reaction paths and molecular vibrations.
- ✓ can calculate and display the true or Multipole Derived Electrostatic Potential and atomic charges can be fitted to the Electrostatic Potential calculated on a Connolly surface.
- ✓ has a powerful Z-matrix editor which give full control over the geometry and allows you to build molecules from scratch, including polypeptides.

G.Schaftenaar and J.H. Noordik, "**Molden**: a pre- and post-processing program for molecular and electronic structures", *J. Comput.-Aided Mol. Design*, 14, 123-134 (2000)

TRITON

A graphical tool for modelling protein mutants and assessment of their activities

•modelling of the protein mutants

The 3D structures of mutants are generated by the **MODELLER program**. This program uses the method of the satisfaction of spatial restrains for model building with the structure from the structural database as a template and the amino acid sequence of the studied protein with the desired substitution as the target sequence.

•preparation of the input data for reaction pathway calculation

Only the active site residues, substrate, co-substrate and co-factors are included in the calculation. In order to mimic the situation in the enzyme, the positions of all backbone atoms are fixed. It requires to create a special file for **MOPAC** which is done by TRITON.

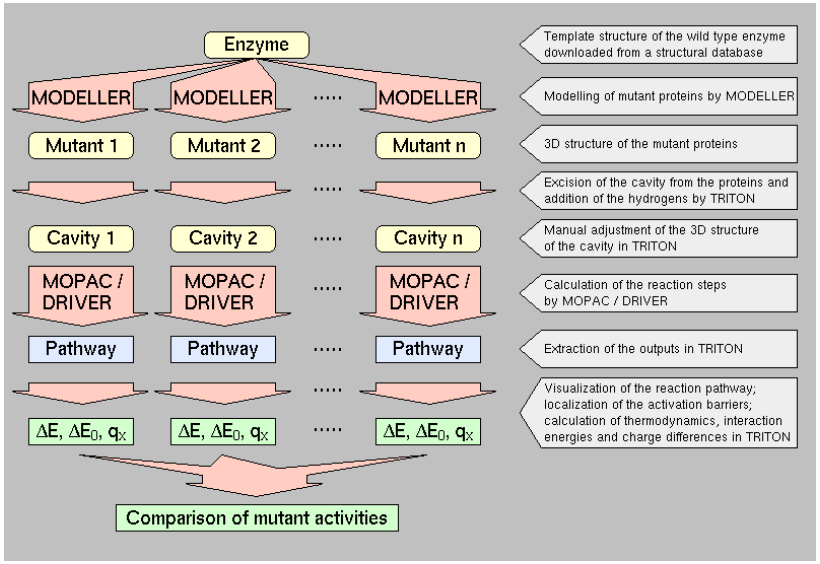
•calculation of the reaction pathways

The reaction pathway is calculated by the semiempirical quantum chemistry program **MOPAC**. The *subroutine DRIVER* is used for reaction pathway mapping. The procedure produces data which can be used to visualise the relationships between the energy and the reaction coordinate, and to estimate the activation barrier and the thermodynamics of the reaction. Changes in partial charges on individual atoms are also monitored during the calculation.

•analysis of the output data

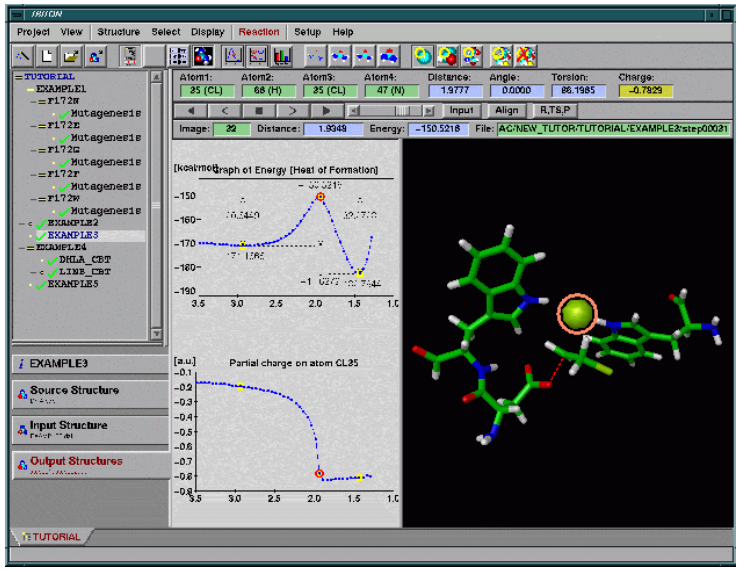
The energies and partial charges on atoms are obtained from output files. The activation barriers of the reaction and changes in partial atomic charges of active site residues during the reaction are then calculated.

TRITON – *Flowchart of the consecutive steps in computational site-directed mutagenesis*



http://ncbr.chemi.muni.cz/triton/

TRITON – Main window



http://ncbr.chemi.muni.cz/triton/

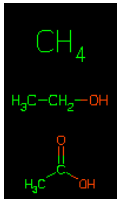
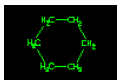
SMILES

Simplified Molecular Input Line Entry Specification

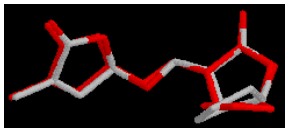
Rules

1. Atoms are represented by atomic symbols: B, C, N, O, F, P, S, Cl, Br, and I.
2. Double bonds are `=', triple bonds are `#'.
3. Branching is indicated by parentheses.
4. Ring closures are indicated by pairs of matching digits.

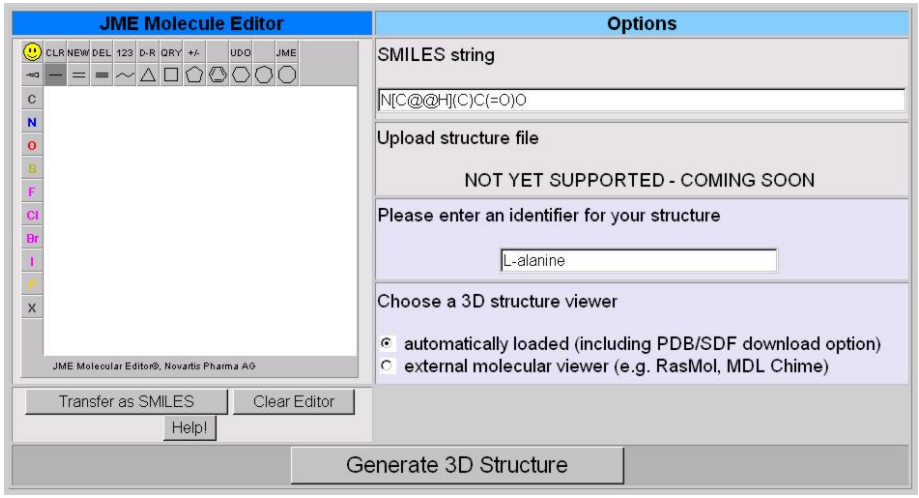
Examples

Depiction	SSMILES	Name	Remark
	C	methane	hydrogens fill normal valence
	CCO	ethanol	a single bond is assumed to join adjacent atoms
	CC(=O)O	acetic acid	parentheses are used to indicate branching
	C1CCCCC1	cyclohexane	bonds can also be represented by pairs of matching digits

Automatic generation of three-dimensional atomic COoRdINAtes



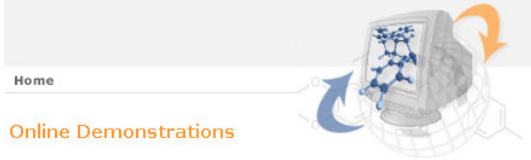
http://www2.chemie.uni-erlangen.de/software/corina/free_struct.html

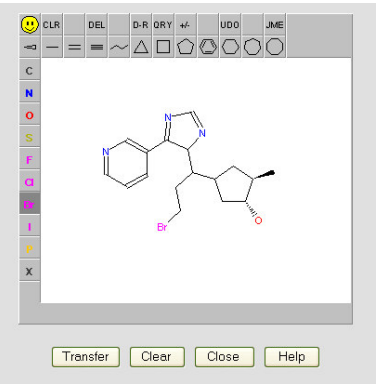


Molecular Networks GmbH
Inspiring Chemical Discovery

Partners

CORINA





Demo - CORINA Interactively

Please enter a structure as SMILES string and an identifier in the form below and press the *Submit* button (or just use "alanin" for demonstration). CORINA will generate 3D coordinates for the given structure. A new page will be generated showing the 3D molecular model if you have RASMOL, CHIME, or some similar program installed on your computer).

C1=CC=NC=C1C2=NC=NC2C(CCBr

Create Molecule

my-molecule

Reset to Demo

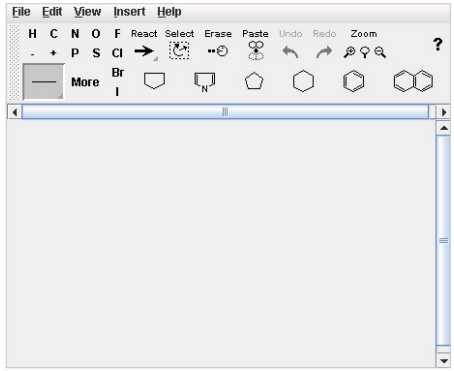
Submit

http://www.molecular-networks.com/online_demos/corina_demo.html

SUPER DRUG DATABASE

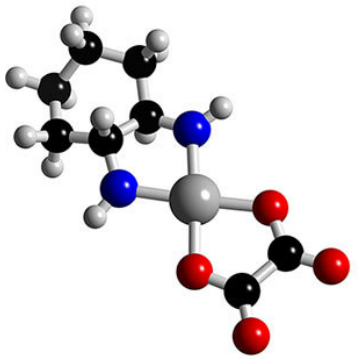
http://bioinformatics.charite.de/superdrug/

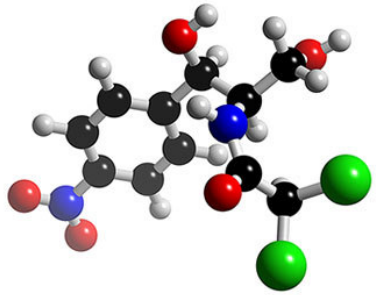
- Home
- Compound Search
- ATC-Classification
- 2D Similarity
- 3D Superposition
- Build your own Structure
- FAQ
- Statistics
- Registration
- Contact
- National Drug Lists
- Links
- Ligand Superpositions



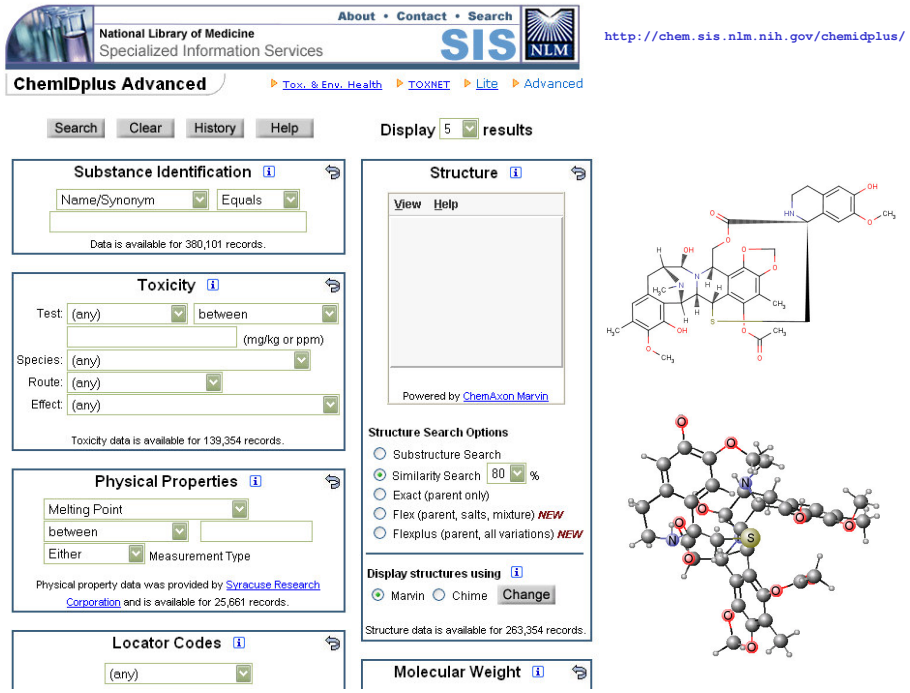
Chemistry, Structures & 3D Molecules @ 3Dchem.com

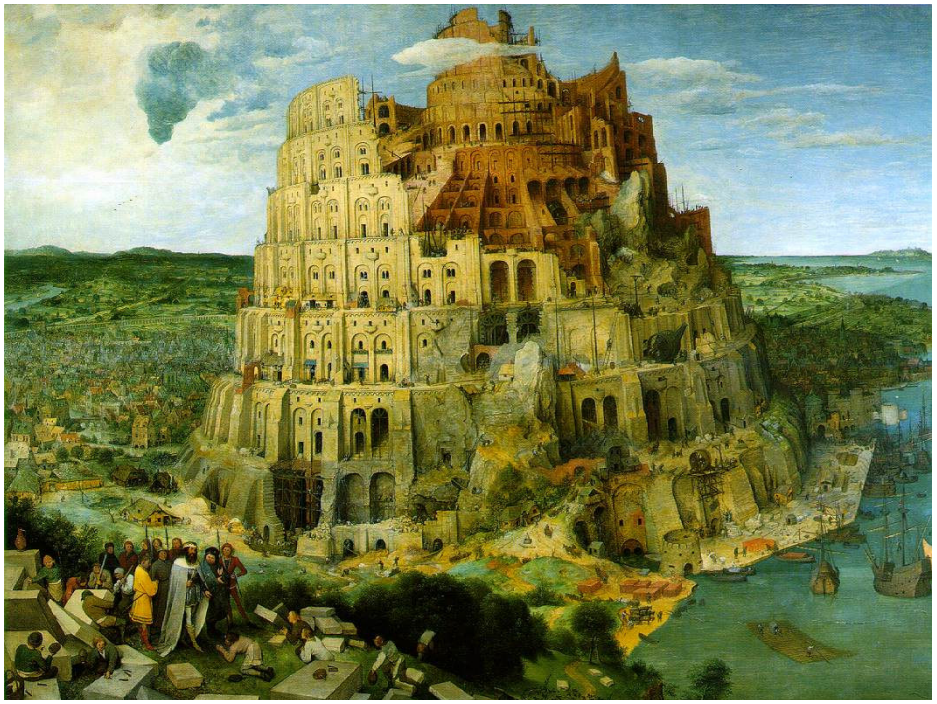
http://www.3dchem.com/atoz.asp





Links: Molecules of the Month, A to Z Index of Structures, Top 50 Prescription Medicines, Gallery, Library of Inorganic Structures (over 1600 structures), Interactive 3D Periodic Table, and Search 3Dchem.com





BABEL A program designed to interconvert a number of file formats currently used in molecular modelling

Input type codes:

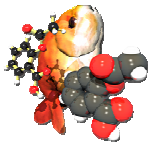
```
alc -- Alchemy file
prep -- AMBER PREP file
bs -- Ball and Stick file
bgf -- MSI BGF file
car -- Biosym .CAR file
boog -- Boogie file
caccrt -- Cacao Cartesian file
cadpac -- Cambridge CADPAC file
charmm -- CHARMm file
c3d1 -- Chem3D Cartesian 1 file
c3d2 -- Chem3D Cartesian 2 file
cssr -- CSD CSSR file
fdat -- CSD FDAT file
gstat -- CSD GSTAT file
dock -- Dock Database file
dpdb -- Dock PDB file
feat -- Feature file
fract -- Free Form Fractional file
gamout -- GAMESS Output file
gzmat -- Gaussian Z-Matrix file
gauout -- Gaussian 92 Output file
g94 -- Gaussian 94 Output file
gr96A -- GROMOS96 (A) file
gr96N -- GROMOS96 (nm) file
hin -- Hyperchem HIN file
sdf -- MDL Isis SDF file
m3d -- M3D file
macmol -- Mac Molecule file
macmod -- Macromodel file
micro -- Micro World file
mm2in -- MM2 Input file
mm2out -- MM2 Output file
mm3 -- MM3 file
mmads -- MMADS file
mdl -- MDL MOLfile file
molen -- MOLIN file
mopcrt -- Mopac Cartesian file
mopint -- Mopac Internal file
mopout -- Mopac Output file
pcmod -- PC Model file
pdb -- PDB file
psin -- PS-GVB Input file
psout -- PS-GVB Output file
msf -- Quanta MSF file
schakal -- Schakal file
shelx -- ShelX file
smiles -- SMILES file
spar -- Spartan file
semi -- Spartan Semi-Empirical file
spmm -- Spartan Mol. Mechanics file
mol -- Sybyl Mol file
mol2 -- Sybyl Mol2 file
wiz -- Conjure file
unixyz -- UniChem XYZ file
xyz -- XYZ file
xed -- XED file
```

BABEL A program designed to interconvert a number of file formats currently used in molecular modelling

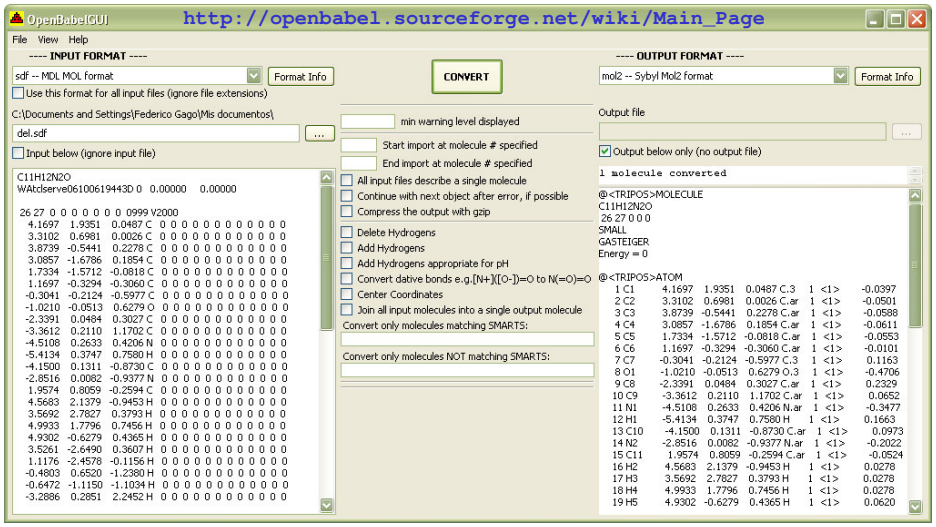
Output type codes:

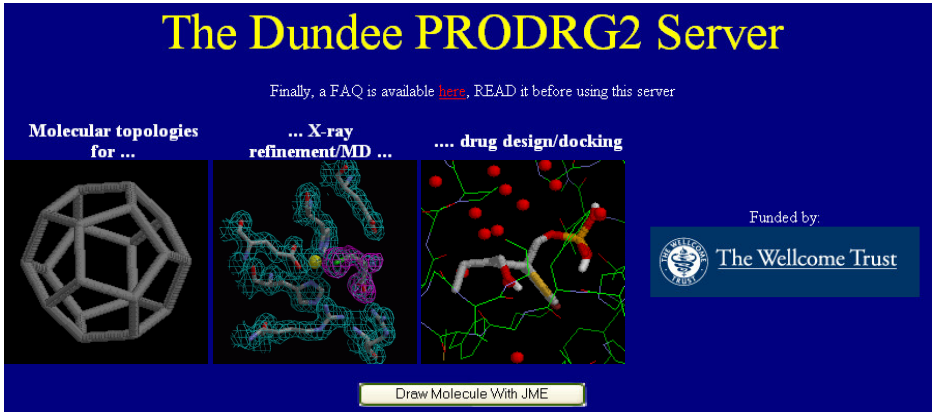
```
diag -- DIAGNOTICS file
t -- Alchemy file
bs -- Ball and Stick file
bmin -- Batchmin Command file
caccrt -- Cacao Cartesian file
cacint -- Cacao Internal file
cache -- CAChe MolStruct file
c3d1 -- Chem3D Cartesian 1 file
c3d2 -- Chem3D Cartesian 2 file
d -- ChemDraw Conn. Table file
con -- Conjure file
contmp -- Conjure Template file
cssr -- CSD CSSR file
feat -- Feature file
fhz -- Fenske-Hall ZMatrix file
gamin -- Gamess Input file
gcart -- Gaussian Cartesian file
g -- Gaussian Z-matrix file
gotmp -- Gaussian Z-matrix tmplt file
hin -- Hyperchem HIN file
icon -- Icon 8 file
i -- IDATM file
macmol -- Mac Molecule file
k -- Macromodel file
micro -- Micro World file
mi -- MM2 Input file
mo -- MM2 Ouput file
mm3 -- MM3 file
mmads -- MMADS file
mdl -- MDL Molfile file
ac -- Mopac Cartesian file
ai -- Mopac Internal file
pc -- PC Model file
p -- PDB file
report -- Report file
spar -- Spartan file
mol -- Sybyl Mol file
mol2 -- Sybyl Mol2 file
maccs -- MDL Maccs file file
xed -- XED file
unixyz -- UniChem XYZ file
x -- XYZ file
```

ftp://ccl.osc.edu/pub/chemistry/software/UNIX/babel/



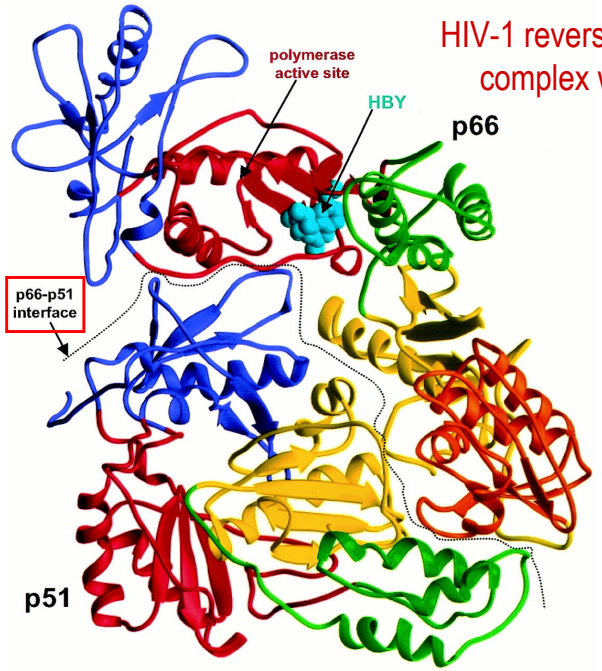
Open Babel is a community-driven scientific project including both cross-platform programs and a developer library designed to support molecular modeling, chemistry, and many related areas, including interconversion of file formats and data.





This WWW PRODRG server will convert coordinates for small molecules in PDB format to the following topology formats: GROMOS, GROMACS, WHAT IF, REFMAC5, CNS, O, SHELX, HEX and MOL2. In addition, coordinates for hydrogen atoms are generated.

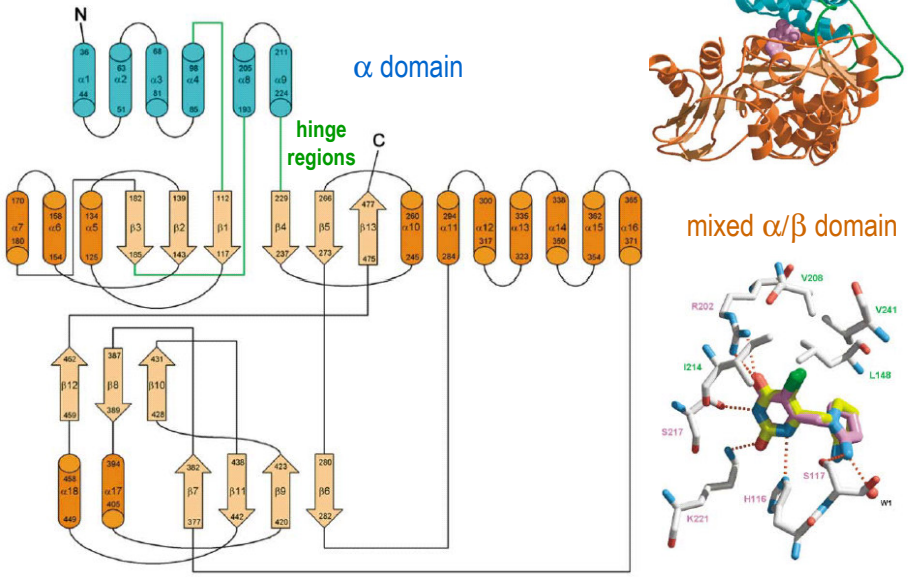
http://davapc1.bioch.dundee.ac.uk/programs/prodrg/



HIV-1 reverse transcriptase in complex with inhibitor HBY

Structure, Vol. 12, 75–84, January, 2004, ©2004 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.str.2003.11.018

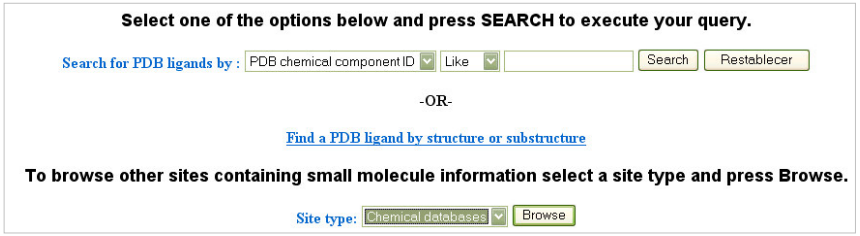
Crystal Structure of Human Thymidine Phosphorylase in Complex with a Small Molecule Inhibitor



Ligand Depot

http://ligand-depot.rutgers.edu/

A data warehouse that integrates databases, services, tools and methods to provide chemical and structural information about the small molecules bound to macromolecules in the Protein Data Bank. It can be used to find codes for existing ligands, link to entries with a particular ligand, and search for substructures.



Ligand Protein Database

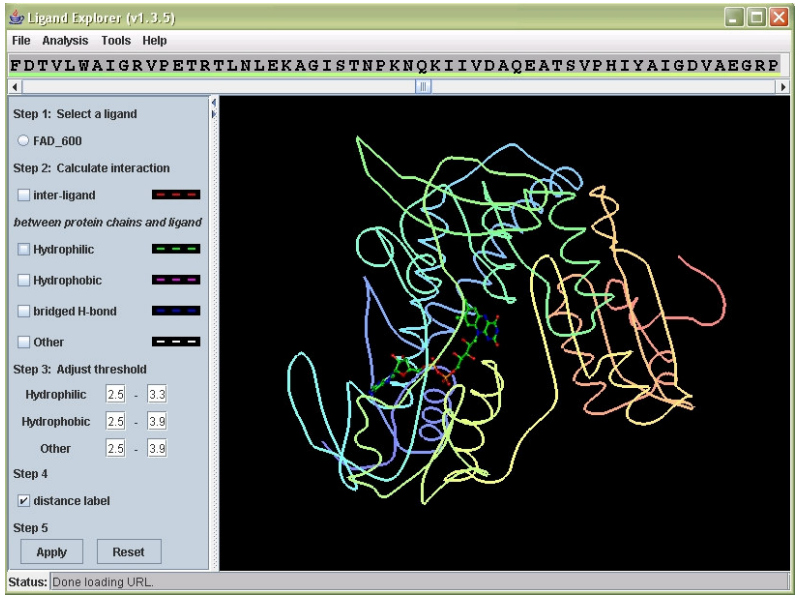
http://lpdb.scripps.edu

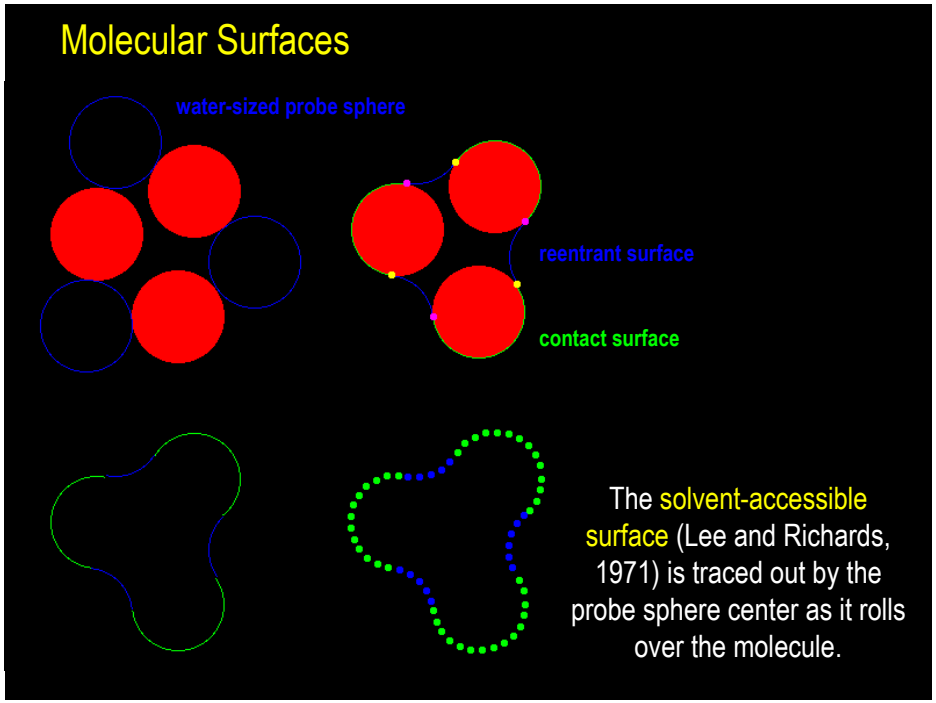
variables	VIP*
number of hydrogen and ionic bonds	1.50
interaction surface area (Å²)	1.31
calculated molecular refractivity (ligand)	1.31
molecular weight (ligand)	1.29
number of atoms (ligand)	1.28
number of donors (ligand)	1.20
number of rotatable bonds (ligand)	1.16
number of acceptors (ligand)	1.14
ClogP (ligand)	1.01

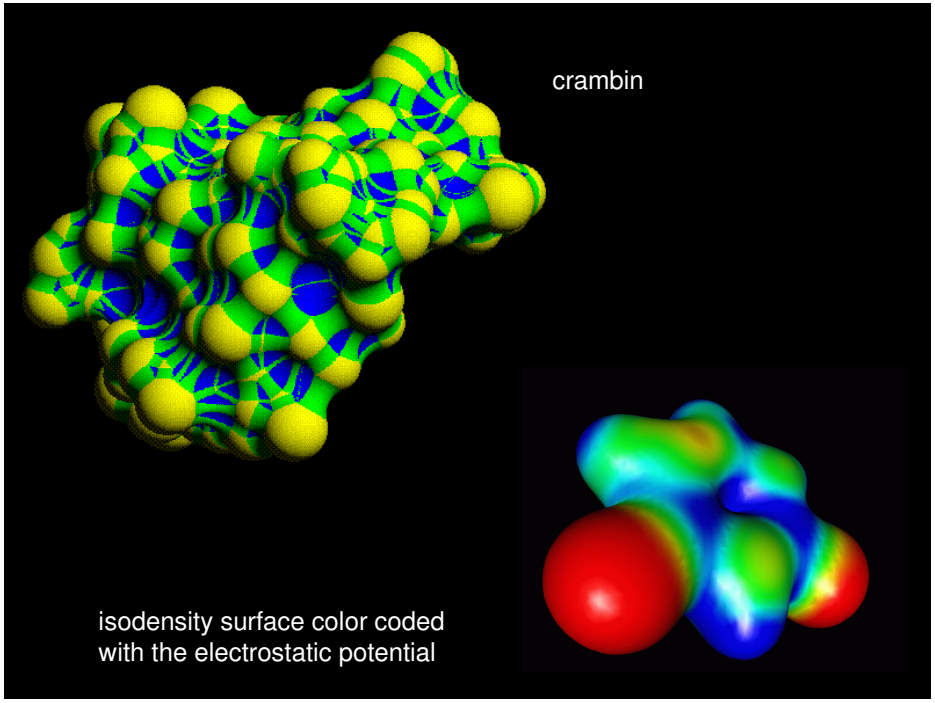
* variable influence on projection parameter

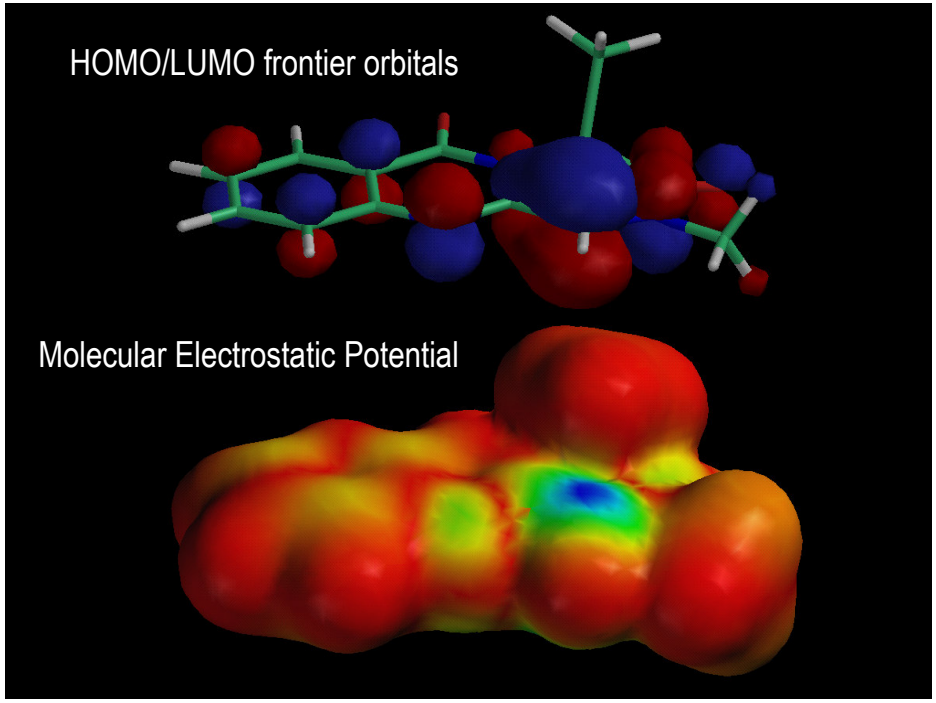
O. Roche, R. Kiyama & C. L. Brooks
J. Med. Chem. **44**, 3592-3598 (2001)

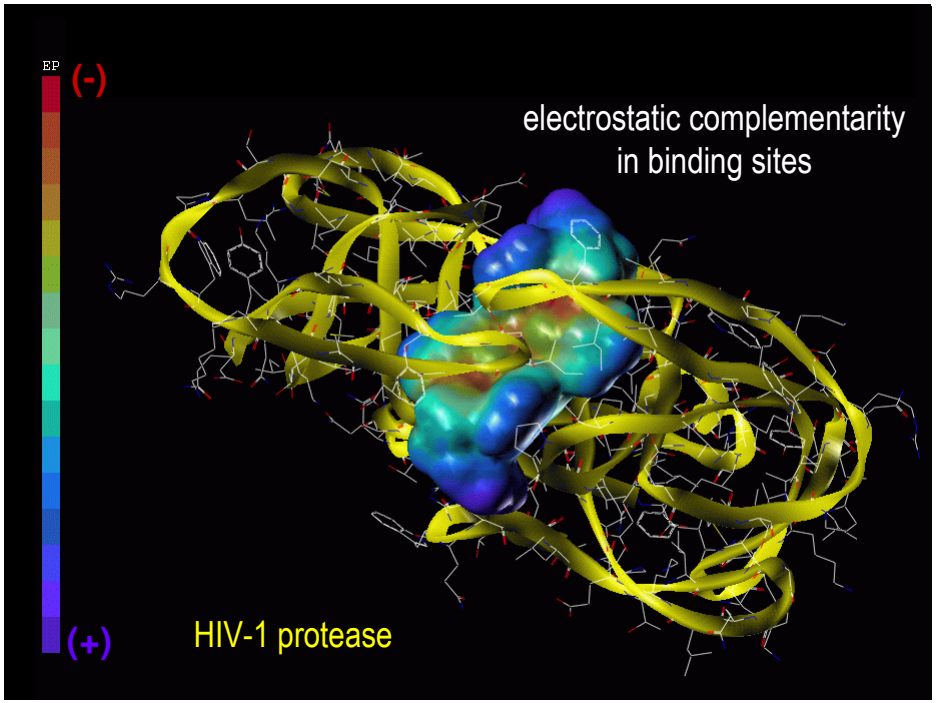
Ligand Explorer











MOLECULAR MECHANICS (MM)

✓ A computational technique used to model the conformational behaviour and energetic properties of molecules.

✓ The molecule is treated at the atomic level, i.e. the electrons are not treated explicitly.

✓ MM uses an Energy Function, defined so that given a particular conformation, (i.e. given a set of spatial coordinates for all the atoms) the energy of the molecule can be calculated.

✓ The energy function is empirical, i.e. it is not entirely derived from rigorous theories.

✓ The energy function makes a distinction between 'bonded' and 'non-bonded' interactions.

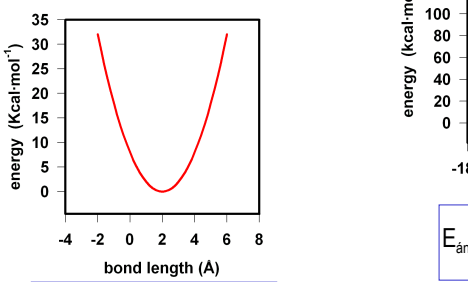
Mecánica Molecular

$$E_{potencial} = E_{enlazada} + E_{no\text{-}enlazada}$$

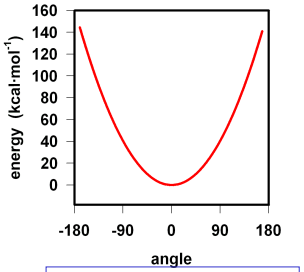
$$E_{enlazada} = \Sigma_i\ E_{enlaces} + \Sigma_i\ E_{ángulos} + \Sigma_i\ E_{diedros}$$

$$E_{no\text{-}enlazada} = \Sigma_i\ E_{electrostática} + \Sigma_i\ E_{van\ der\ Waals}$$

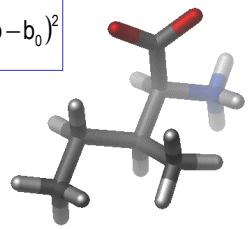
TÉRMINOS ENLAZADOS

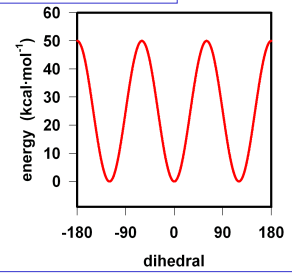


$$E_{enlaces} = \sum_{enlaces} \frac{1}{2} k_b (b - b_0)^2$$



$$E_{ángulos} = \sum_{ángulos} \frac{1}{2} k_\theta (\theta - \theta_0)^2$$

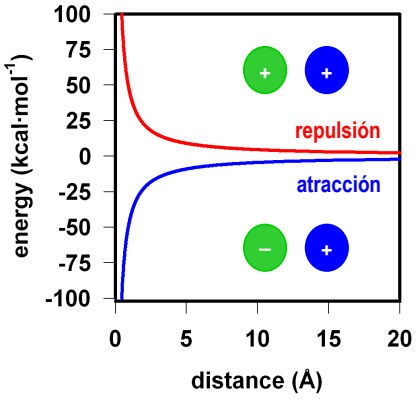




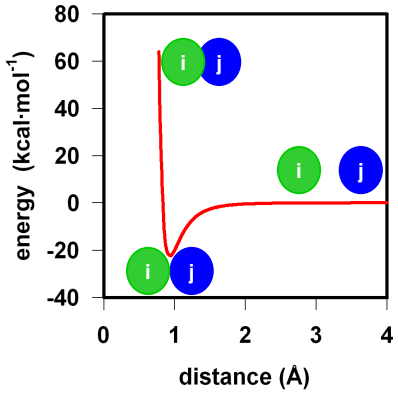
$$E_{dihedros} = \sum_{dihedros} \frac{1}{2} k_d [1 + \cos(\varphi - \varphi_0)]$$

TÉRMINOS NO-ENLAZADOS

$$E_{electrostática} = \frac{1}{4\pi\pi\varepsilon_0\varepsilon} \sum_{ij} \frac{q_i q_j}{r_{ij}}$$



$$E_{Lennard\text{–}Jones} = \sum_{ij} \frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}$$



Empirical Potential Energy Function

Bonds

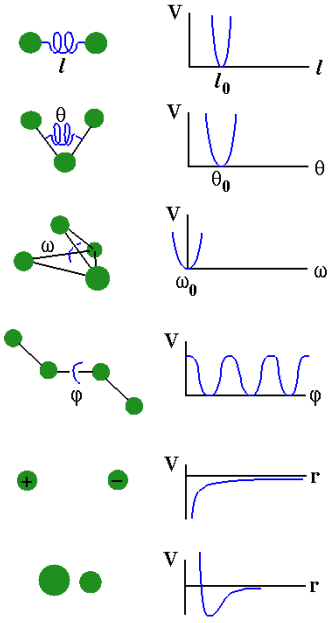
Angles

Improper Dihedrals

Torsions

Electrostatics

van der Waals

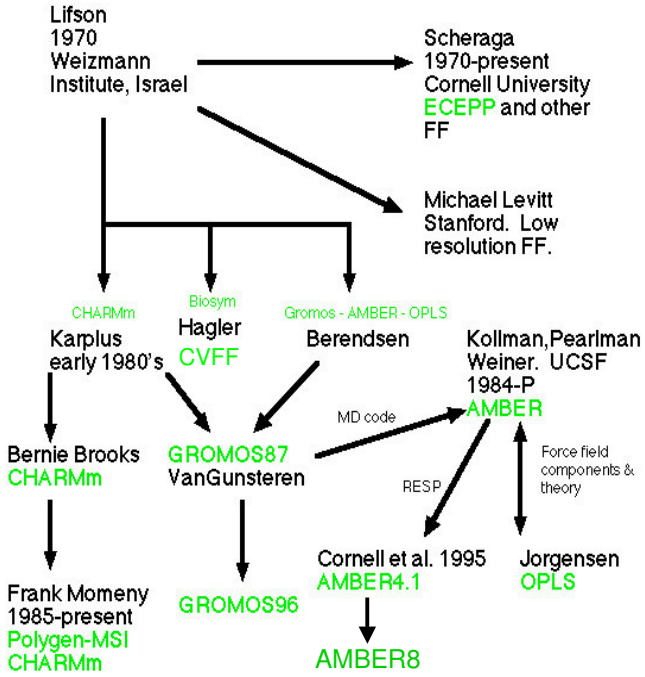


Resumen de interacciones incluidas en un **campo de fuerzas** representativo de mecánica molecular

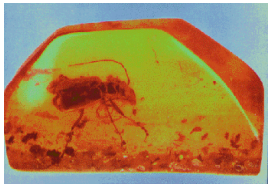
$$V = \sum_{enlaces} k_b (b - b_0)^2 + \sum_{ángulos} k_\theta (\theta - \theta_0)^2 +$$
$$+ \sum_{diedros} \sum_{n=1}^{N} K_\phi^{(n)} [1 + \cos(n\phi - \delta)] + \sum_{impropios} K_\omega (\omega - \omega_0)^2$$
$$+ \sum_{i,j} 4\varepsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \sum_{i,j} \left(\frac{q_i q_j}{D r_{ij}} \right)$$

✓ La función empírica de energía potencial es diferenciable con respecto a las coordenadas atómicas.

✓ Esto proporciona el valor y la dirección de la fuerza que actúa sobre cada átomo y puede así utilizarse en una simulación de dinámica molecular.



AMBER (Assisted Model Building with Energy Refinement)



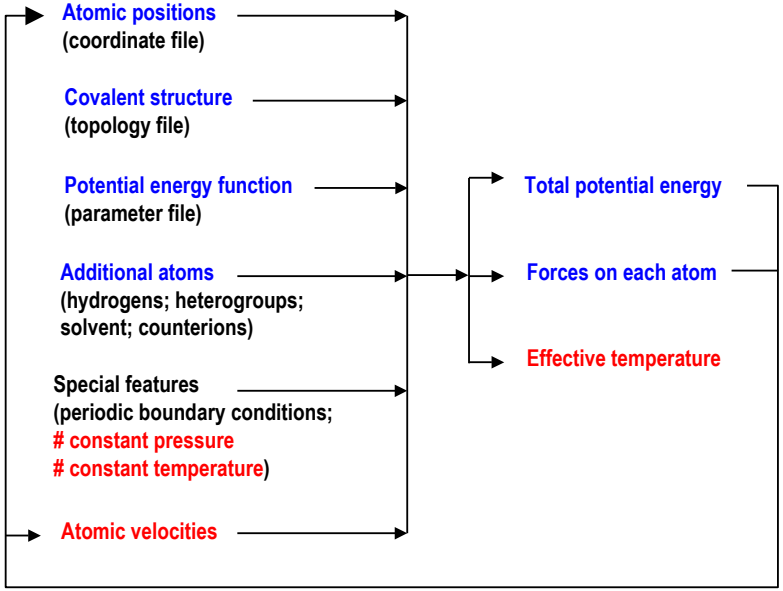
CHARMm® (Chemistry at HARvard Macromolecular Mechanics)

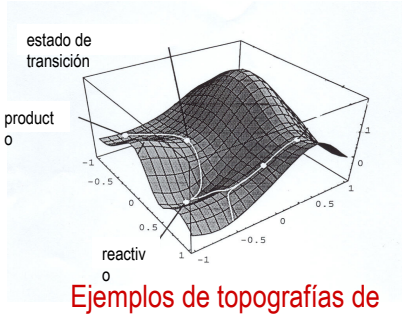
CVFF (Consistent-Valence Force Field)

GROMOS (GROningen MOlecular Simulation package)

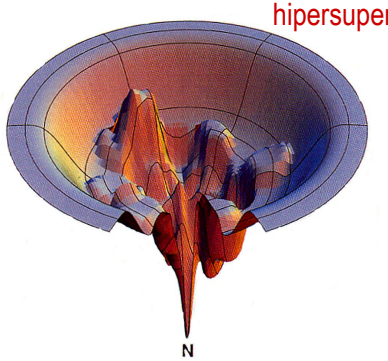
OPLS (Optimized Potentials for Liquid Simulations)

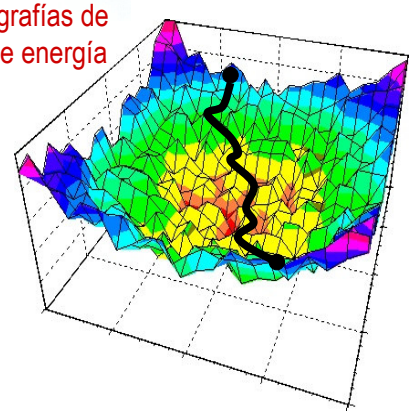
ALGORITHMS FOR **ENERGY MINIMIZATION** AND **MOLECULAR DYNAMICS**





Ejemplos de topografías de hipersuperficies de energía





Dinámica Molecular

Segunda ley de Newton:

$$-\frac{dE}{dx} = F = m \cdot a =$$

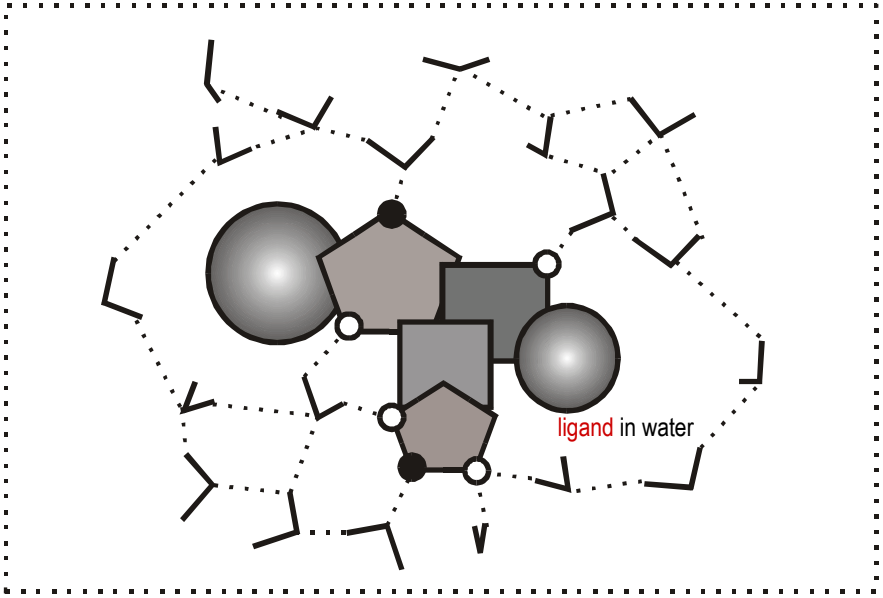
$$m \cdot \frac{v}{t} = m \cdot \frac{d^2x}{dt^2}$$

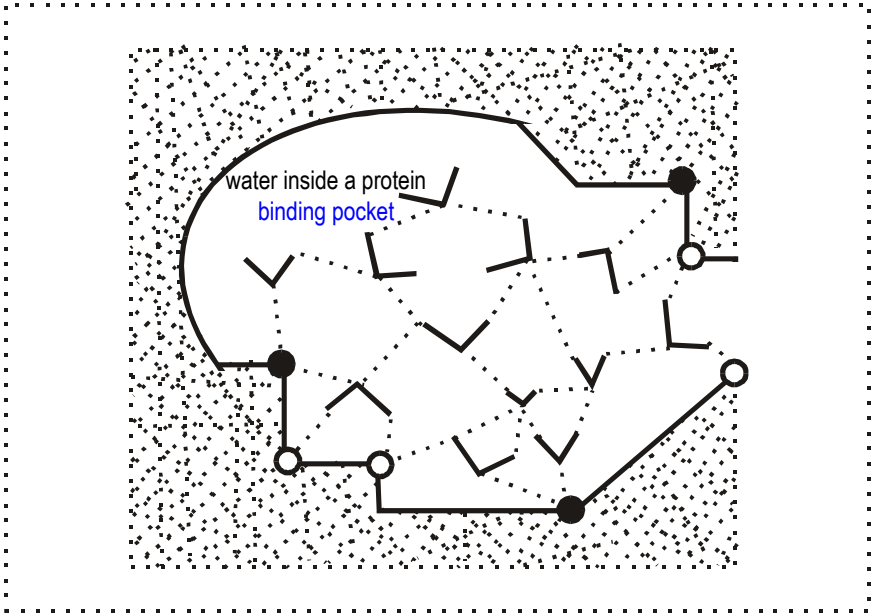
Las simulaciones de Dinámica Molecular permiten el estudio de procesos dinámicos complejos que ocurren en los sistemas biológicos, por ejemplo:

- ✓ Estabilidad de proteínas
- ✓ Cambios conformacionales
- ✓ Plegamiento de proteínas
- ✓ Reconocimiento molecular: ligandos, proteínas, ADN...
- ✓ Transporte de iones en sistemas biológicos

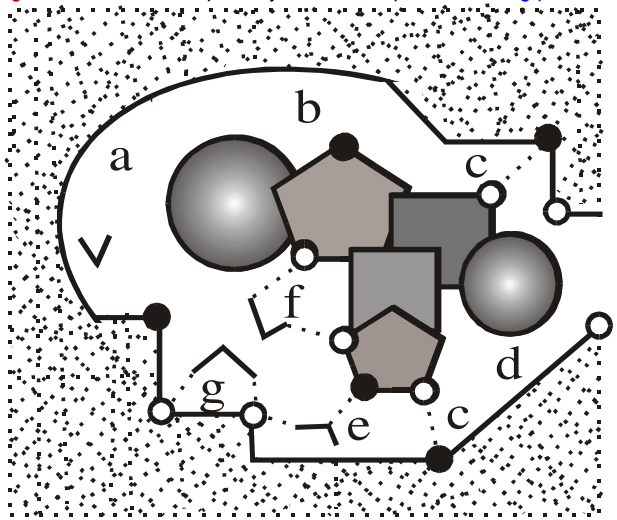
y proporcionan un medio valioso para llevar a cabo estudios de:

- determinación de estructuras por difracción de rayos X y espectroscopía de RMN
- diseño de nuevos fármacos



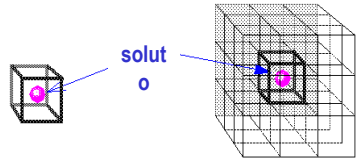


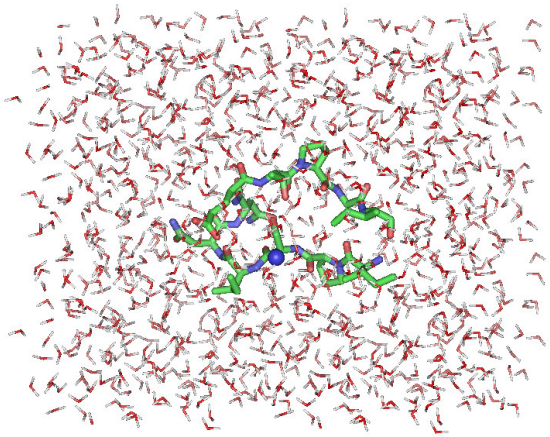
ligand buried inside a partially desolvated protein binding pocket



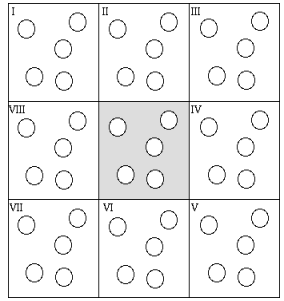
Conclusion: Any comprehensive method that attempts to model ligand binding must also consider the energy of solvation and entropic contributions to the binding process.

TRATAMIENTO EXPLÍCITO DEL DISOLVENTE

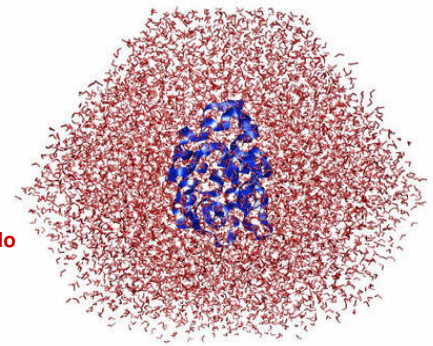




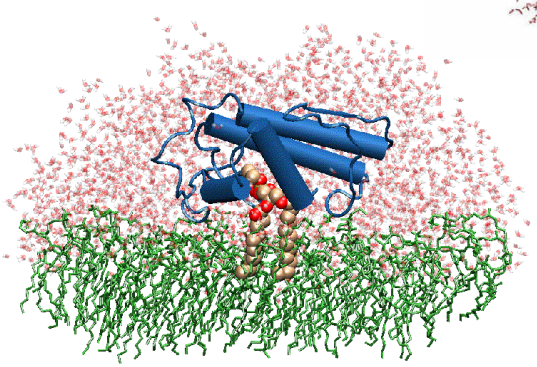
CONDICIONES DE LÍMITE PERIÓDICO

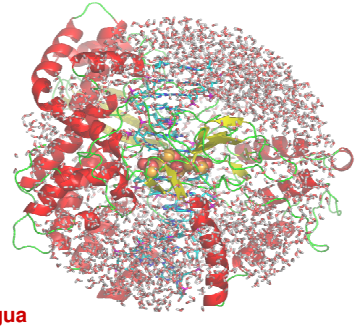


TRATAMIENTO EXPLÍCITO DEL DISOLVENTE

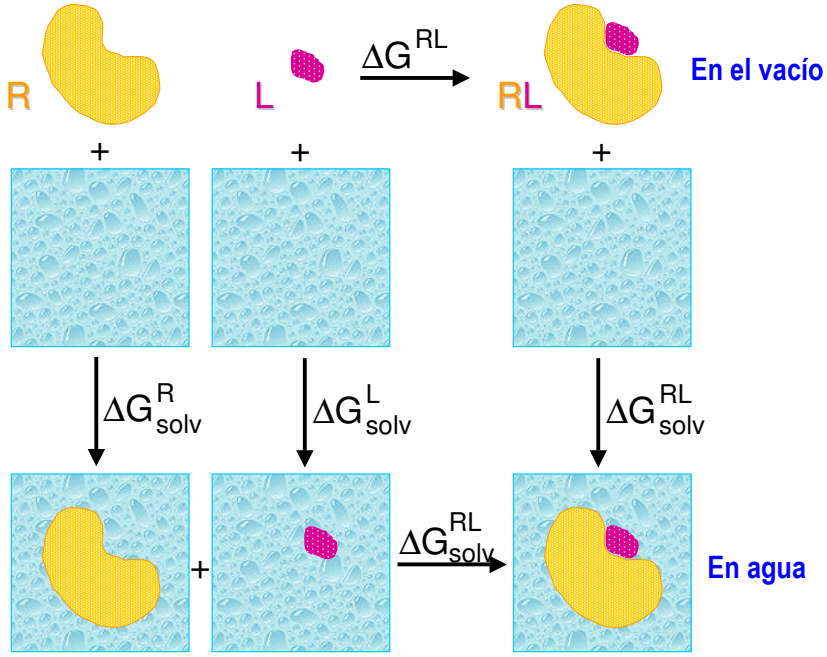


octaedro truncado





"gotas" o "conchas" de agua



"DelPhi - A Macromolecular Electrostatics Modelling Package":

Kim A. Sharp, Anthony Nicholls & Barry Honig
Department of Biochemistry and Molecular Biophysics, Columbia University, New York

- Klapper, I.; Hagstrom, R.; Fine, R.; Sharp, K.; Honig, B. "Focusing of Electric Fields in the Active Site of Cu-Zn Superoxide Dismutase: Effects of Ionic Strength and Amino-acid Modification." ***Proteins (1986) 1, 47-59.***

- Gilson, M. K.; Sharp, K. A.; Honig, B. H. "Calculating the Electrostatic Potential of Molecules in Solution: Method and Error Assessment" ***J. Comput. Chem. (1987) 9, 327-335.***

- Gilson, M. K.; Honig, B. "Calculation of the Total Electrostatic Energy of a Macromolecular System: Solvation Energies, Binding Energies, and Conformational Analysis." ***Proteins (1988) 4, 7-18.***

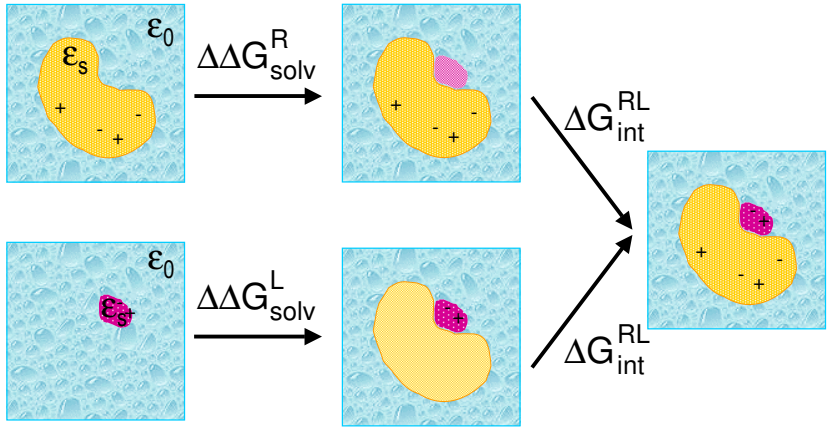
- K. Sharp, K.; Honig, B. "Electrostatic Interactions in Macromolecules: Theory and Applications." ***Ann. Rev. Biophys. Biopys. Chem. (1990) 19, 301-332.***

- Nicholls, A.; Honig, B. "A Rapid Finite Difference Algorithm, Utilizing Successive Over-Relaxation to Solve the Poisson-Boltzmann Equation." ***J. Comput. Chem. (1991) 12, 435-445.***

The original reference to the use of the finite difference method for macromolecular electrostatics is: J. Warwicker and H. C. Watson, *J. Mol. Biol. (1982) 157, 671.*

Ecuación de Poisson: $\nabla^2\phi(r) = -\frac{4\pi\rho(r)}{\varepsilon}$

Ecuación de Poisson-Boltzmann: $\nabla\cdot[\varepsilon(r)\nabla\phi(r)] - k'\phi(r) = -4\pi\rho(r)$



Affinity *vs.* Specificity

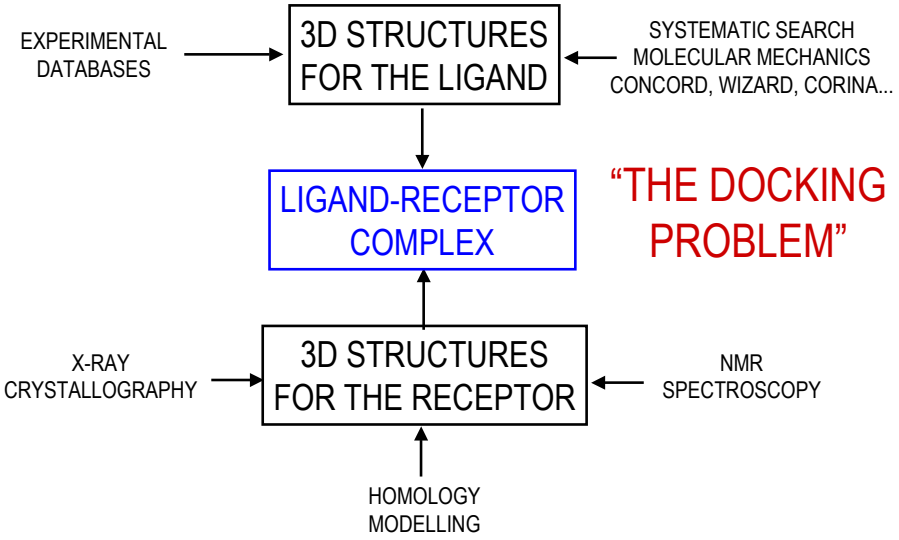
$$\text{Ligand} + \text{Receptor} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{Ligand-Receptor}$$

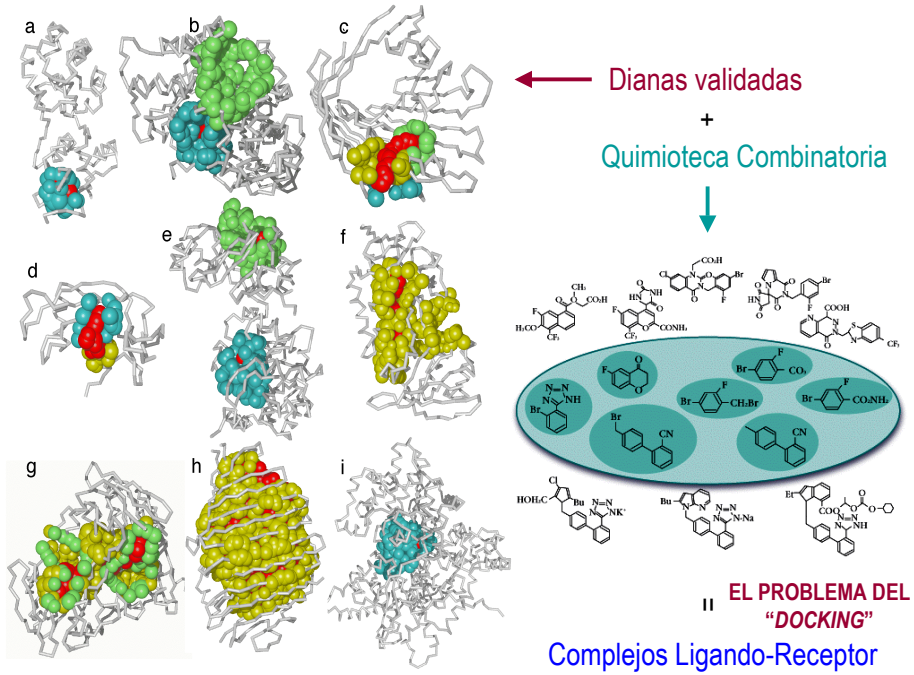
$$K_d = \frac{k_{off}}{k_{on}} = \frac{[\text{Ligand}]\,[\text{Receptor}]}{[\text{Ligand-Receptor}]}$$

$$\Delta G = \Delta H - T\Delta S$$

Binding constant ΔK_d	**Binding energy** ΔG (kcal/mol)
2x	0.5
5x	1.0
13x	1.5
29x	2.0
68x	2.5
158x	3.0

$$\Delta G = 2.303\ RT \log K_d$$



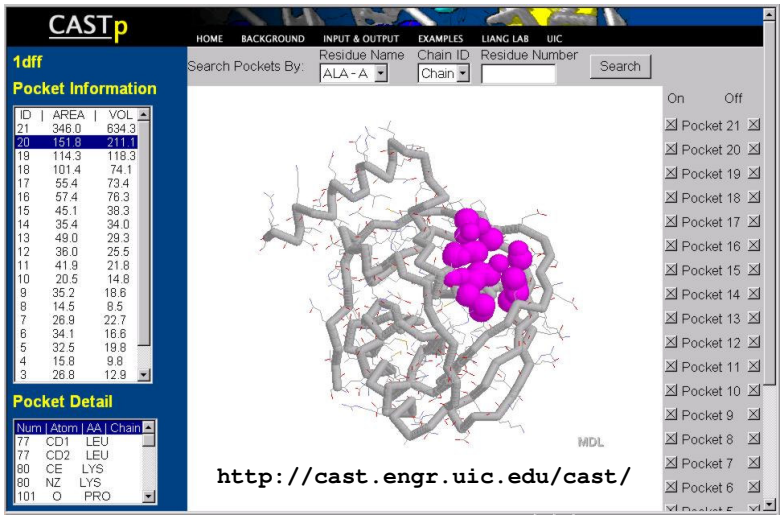


II **EL PROBLEMA DEL *"DOCKING"***

Complejos Ligando-Receptor

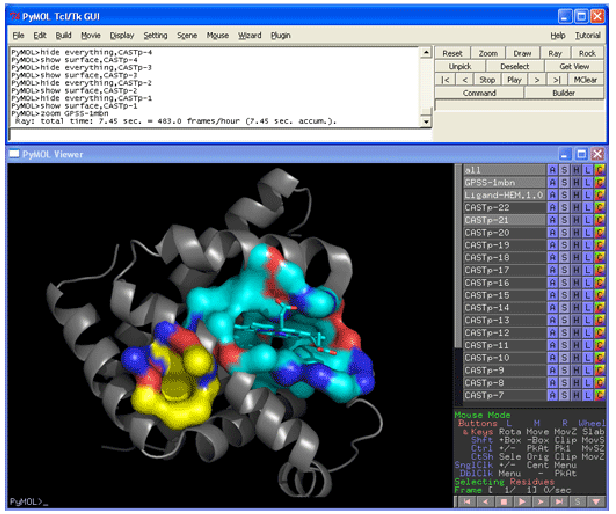
CASTp — *A Server for Identification of Protein Pockets & Cavities*

- Identifies all pockets and cavities.
- Measures the volume and area analytically.



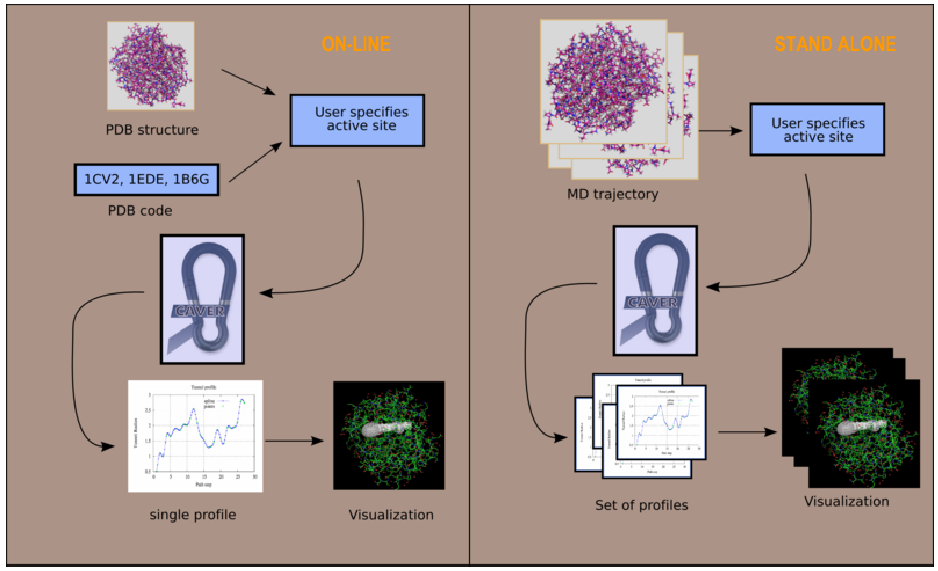
CASTp — *A Server for Identification of Protein Pockets & Cavities*

- GPSSpyMOL: Global Protein Surface Survey Plugin for PyMOL



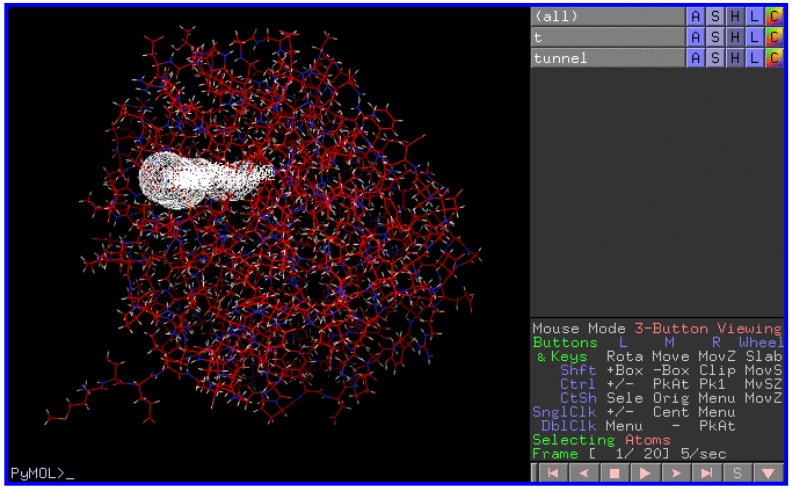
CAVER ... secure caving in the world of biomolecules

http://loschmidt.chemi.muni.cz/caver/

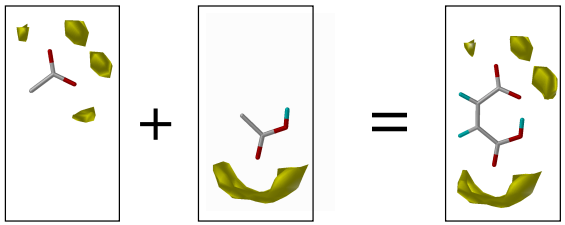


http://loschmidt.chemi.muni.cz/caver/

Changes of shape in the access tunnel (white mesh) to the active site during a MD trajectory



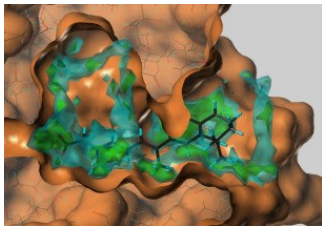
SuperStar



- Calculate binding positions for specific probe atoms in protein active sites
- Identify functional groups in binding-site
- Look up relevant IsoStar scatterplots and overlay on functional groups
- Contour - combining by taking products

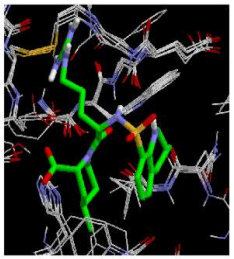
SuperStar Features

map for aromatic CH carbon probe generated at the binding site of the protein-ligand complex 1CPS.



- Cavity detection
- Surface or pharmacophore point display
- Metal coordination
- Hyperlinking to IsoStar scatterplots
- Choice of CSD- or PDB-based maps
- Gaussian fits

Relibase



Relibase: A program for searching protein-ligand databases.

Version 4.0, October 2000

Install 3D visualization software

Comments/bugs/queries to:

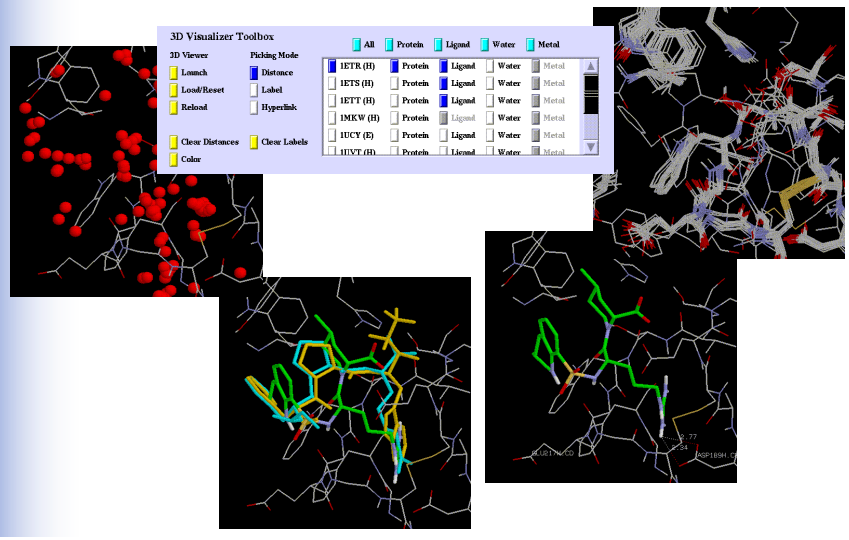
Cambridge Crystallographic Data Centre
12 Union Road
Cambridge CB2 1EZ
United Kingdom
tel: (44) (1223) 336022
e-mail: support@ccdc.cam.ac.uk

http://relibase.ccdc.cam.ac.uk/

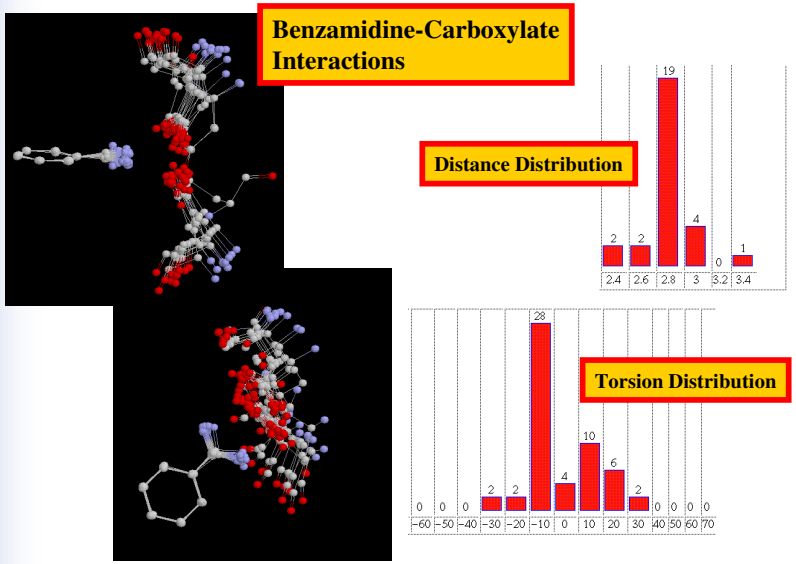
http://relibase.ebi.ac.uk/

http://relibase.rutgers.edu/

Binding Site Superposition



Analysis of 3D Queries



Relibase+

- Protein-ligand database system
- Based on original software developed by Manfred Hendlich and colleagues at Merck and Marburg University
- Enables searching of PDB and of in-house proprietary databases

Relibase: **http://relibase.ccdc.cam.ac.uk/**

Some Relibase+ Options

- Text searching
- Sequence searching
- 2D substructure and similarity searching
- 3D substructure searching
- Logical combination of hit lists
- Searching for intermolecular interactions
- Auto-superposition of similar binding sites
- Scripting facility based on Python

LIGPLOT

http://www.biochem.ucl.ac.uk/bsm/ligplot/ligplot.html

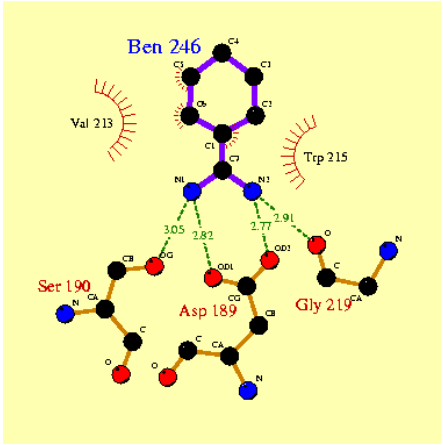
Program for automatically plotting protein-ligand interactions (by A. Wallace & R. Laskowski)

Automatically generates schematic diagrams of protein-ligand interactions for a given PDB file.

hydrogen bonds: dashed lines between the atoms involved.

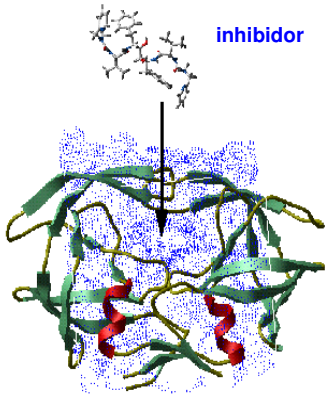
hydrophobic contact: an arc with spokes radiating towards the ligand atoms they contact. The contacted atoms are shown with spokes radiating back.

Atom accessibilities can also be depicted; the ligand atoms can be colour-coded to indicate their accessibility to solvent.

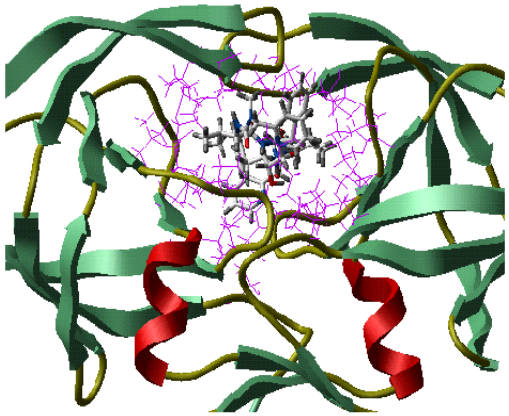


benzamidine (PDB code 2TBS)

ACOPLAMIENTO LIGANDO-RECEPTOR (*DOCKING*



Proteasa de VIH-1



Complejo enzima-inhibidor

Virtual ("in silico") screening

Docking/scoring programs

Docking engines: search the conformational space in the binding site

Scoring functions: discrimination of correctly docked from misdocked conformations

MOLECULAR DOCKING

- SYSTEMATIC SEARCH (*brute force algorithm*):

All binding orientations of all conformers of the ligand and the receptor (impractical for most situations).

- AUTOMATED SEARCH:

GEOMETRIC METHODS: Matching of ligand and receptor site descriptors (descriptors, grids, fragments...).

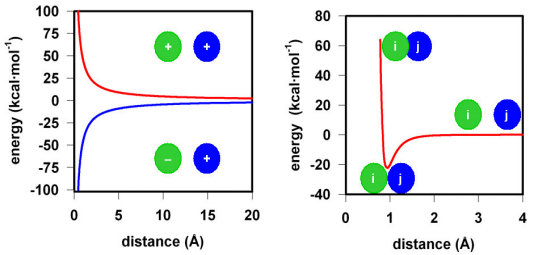
FORCE FIELD METHODS: Minimizing the ligand-receptor interaction energy - Molecular dynamics and Monte Carlo simulations.

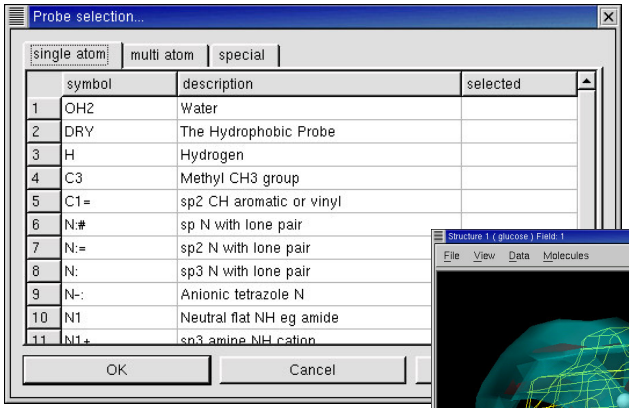
"GRID: A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules"

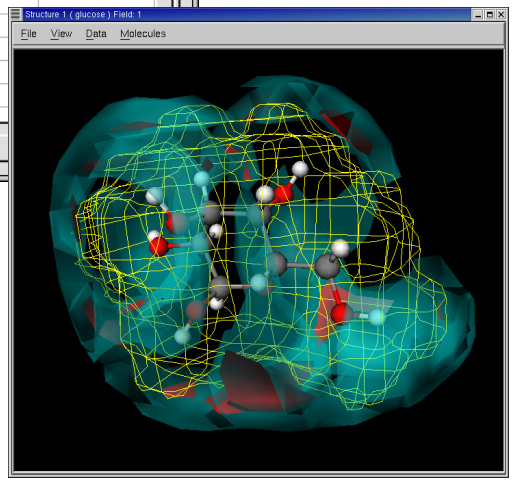
Peter Goodford, Oxford University

J. Med. Chem. *28,* 849-857 (1985)
ibid. *32,* 1083-1094 (1989); *36,* 140-147 (1993); *36,* 148-156 (1993)

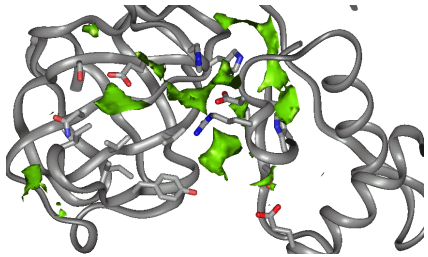
http://www.moldiscovery.com/



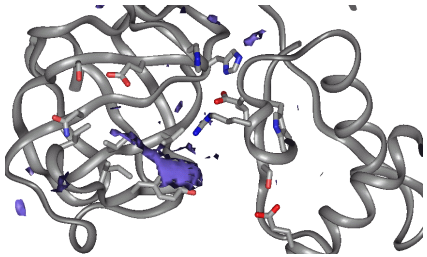




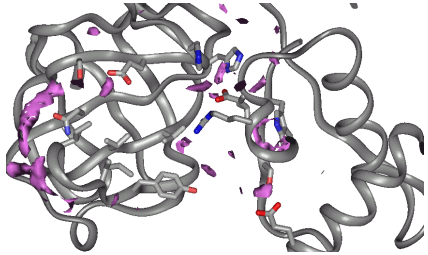
http://www.moldiscovery.com/



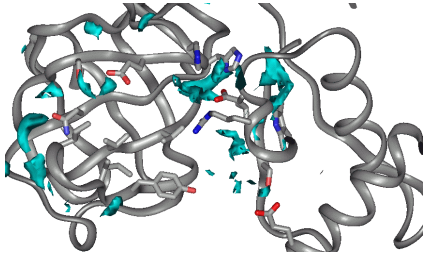
Aromatic carbon probe
Grid point value range: -5.45 to 5.0 kcal/mol
Contour level: -2.5 kcal/mol



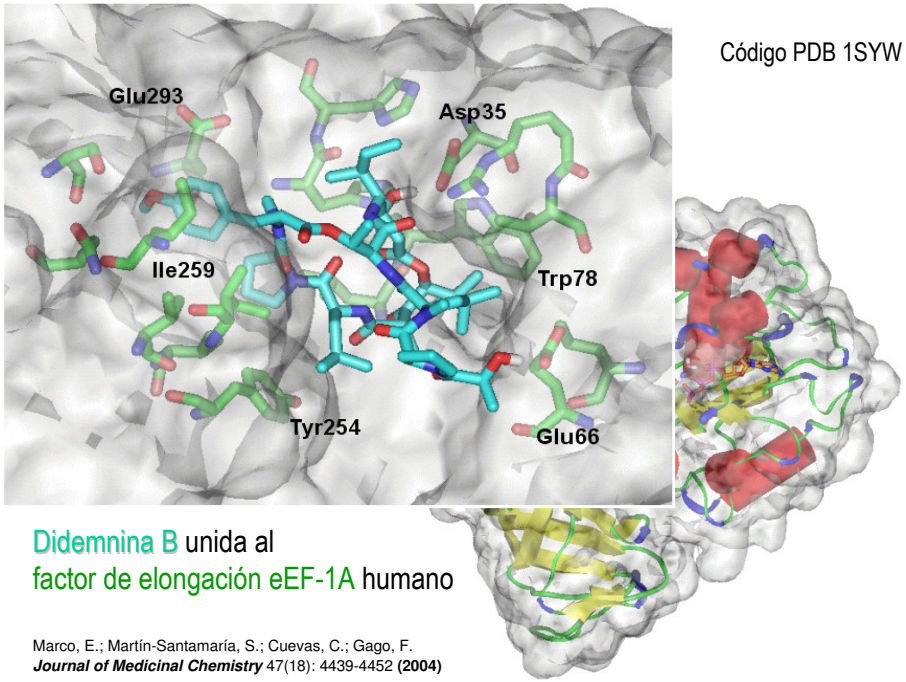
Hydrophobic probe
Grid point value range: -2.86 to 0.0 kcal/mol
Contour level: -1.0 kcal/mol



Carbonyl oxygen probe
Grid point value range: -8.03 to 5.0 kcal/mol
Contour level: -5.0 kcal/mol



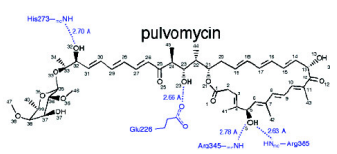
Hydroxyl oxygen probe
Grid point value range: -12.30 to 5.0 kcal/mol
Contour level: -7.0 kcal/mol

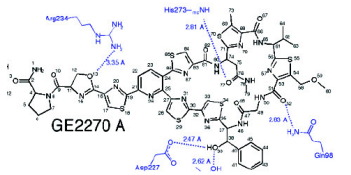


Código PDB 1SYW

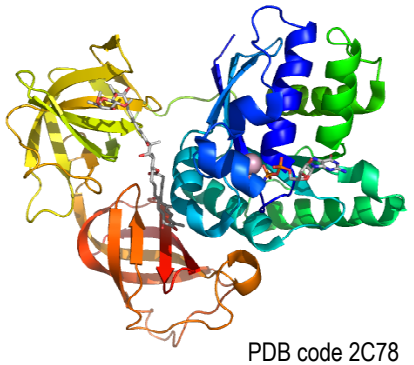
Didemnina B unida al
factor de elongación eEF-1A humano

Marco, E.; Martín-Santamaría, S.; Cuevas, C.; Gago, F.
Journal of Medicinal Chemistry 47(18): 4439-4452 **(2004)**

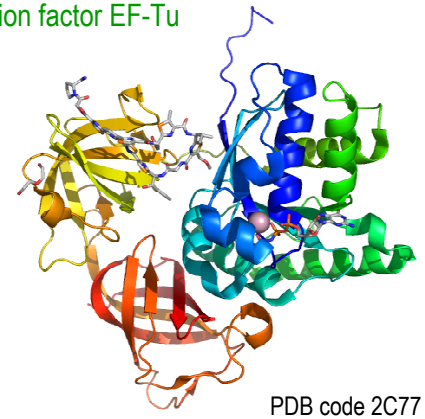




Prokaryotic elongation factor EF-Tu



PDB code 2C78



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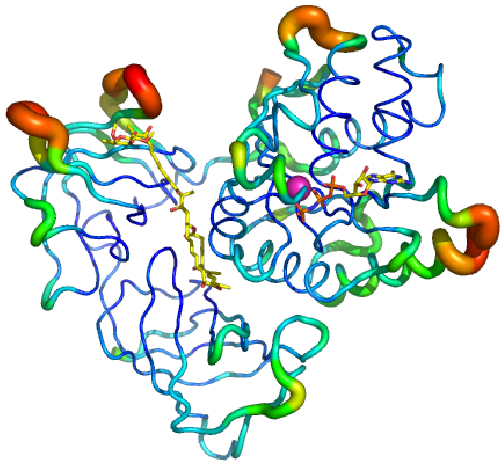


Why Use Molecular Docking?

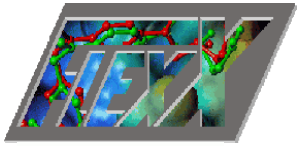
- Most detailed representation of binding site
  - overcomes simplifications of pharmacophores
  - identifies both conservative and novel solutions
  - provides impetus for *de novo* design/optimisation
- Broad range of analyses applicable
  - diverse scoring/selection criteria
- Quality/throughput of available methods
  - good enough, despite technical limitations

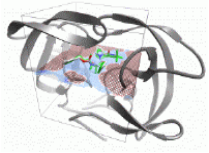
"THE DOCKING PROBLEM"

- SITE/LIGAND REPRESENTATION (treatment of H atoms?)
- JUXTAPOSITION OF THE LIGAND AND SITE FRAMES OF REFERENCE (docking engine)
- EVALUATION OF COMPLEMENTARITY (scoring functions)

AIM: To obtain the lowest free energy structure(s) for the receptor-ligand complex

Examples of docking algorithms





Rigid ligand:

Fast shape matching (DOCK)

Flexible ligand:

Fast shape matching (DOCK 4.0)

Incremental construction (FlexX)

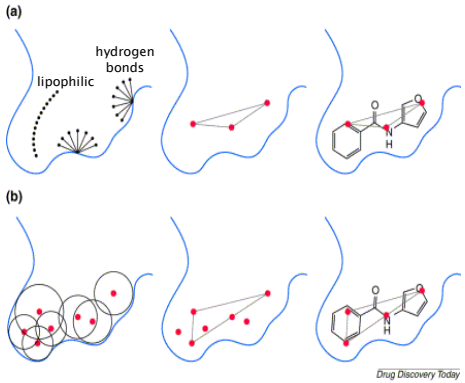
Simulated annealing (AutoDock 2.4)

Monte Carlo simulations (MCDOCK)

Genetic algorithm (AutoDock 3.0, GOLD, GAMBLER)

FlexX algorithm

DOCK algorithm



surface of the receptor pocket

FlexX matches triangles of interaction sites onto complementary ligand atoms.

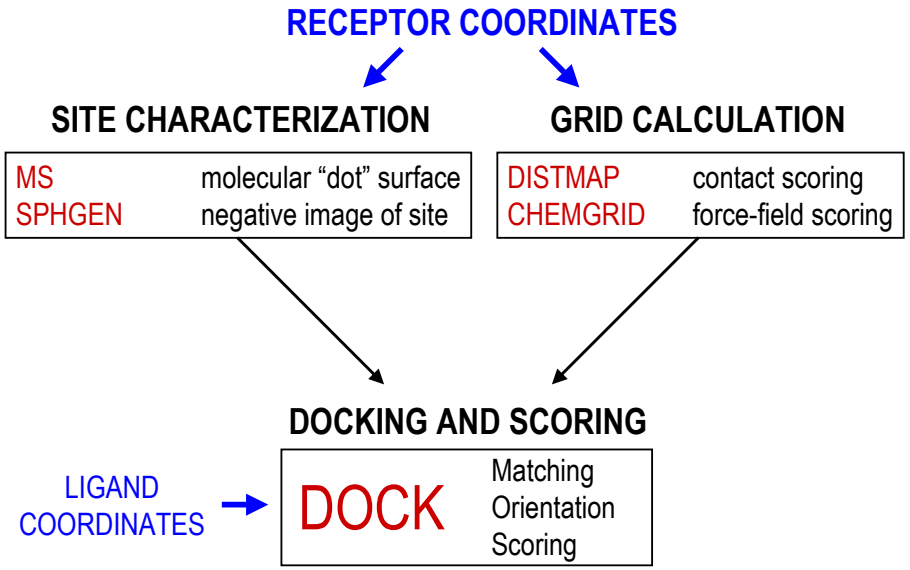
DOCK fills the binding site with spheres, and sphere centers are then matched to the ligand atoms to determine plausible ligand–receptor complexes.

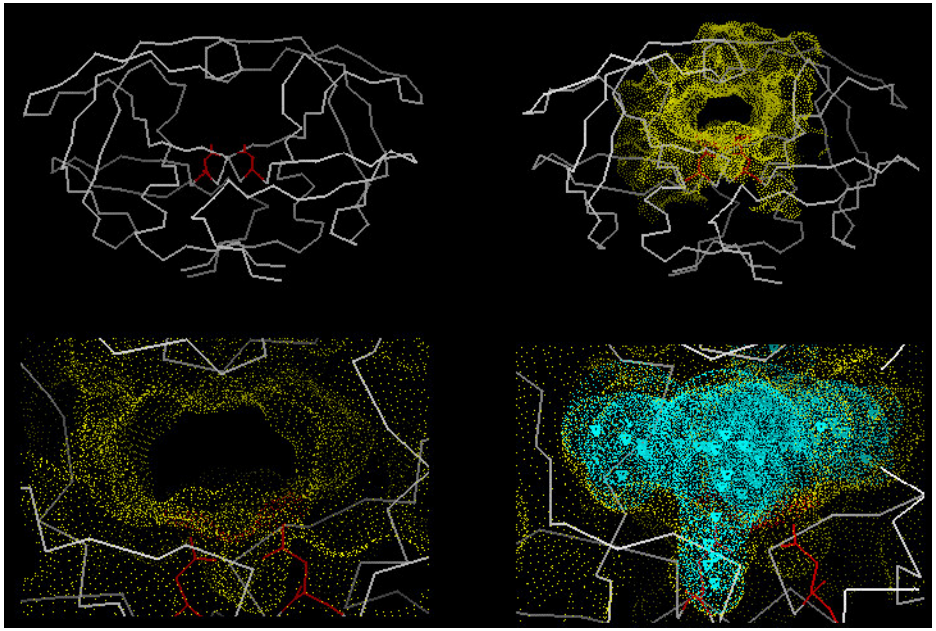
PROGRAM **DOCK**

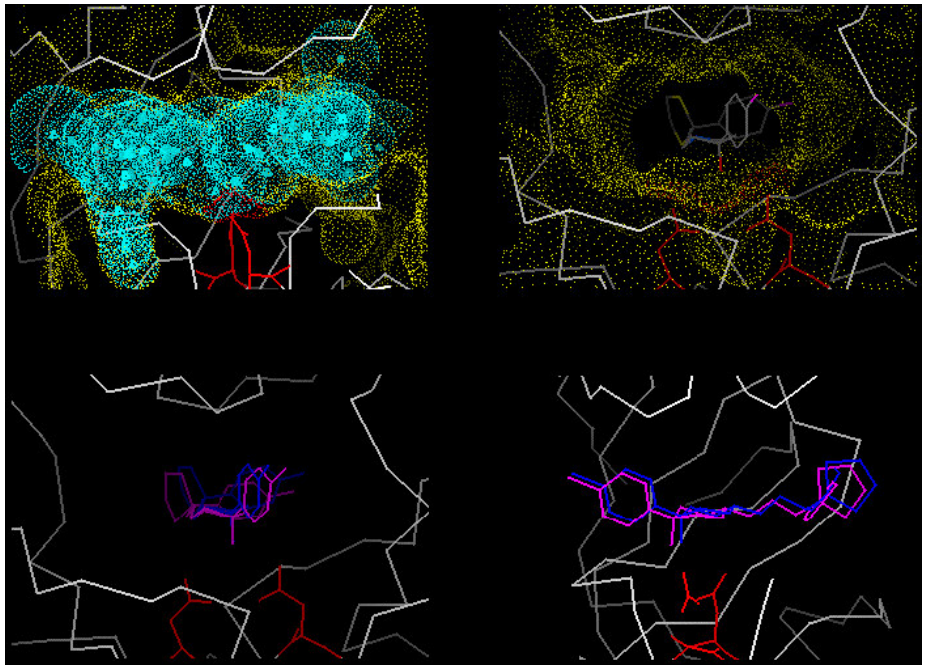
"A Geometric Approach to Macromolecule-Ligand Interactions"
I. D. Kuntz, J. M. Blaney, S. J. Oatley, R. Langridge, T. E. Ferrin
J. Mol. Biol. 161, 269-288 (1982)

"Using Shape Complementarity as an Initial Screen in Designing Ligands for a Receptor Binding Site of Known Three-Dimensional Structure"
R. L. DesJarlais, R. P. Sheridan, G. L. Seibel, J. S. Dixon, I. D. Kuntz, R. Venkataraghavan
J. Med. Chem. 31, 722-729 (1988)

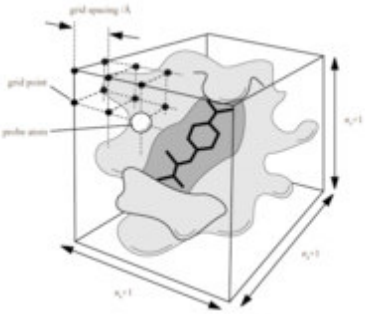
"Automated Docking with Grid-Based Energy Evaluation"
E. C. Meng, B. K. Soichet, I. D. Kuntz
J. Comp. Chem. 13, 505-524 (1991)





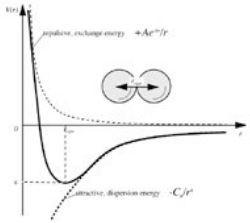


AutoDock: Why Use Grid Maps?



- AutoGrid computes grid maps
  - Representation of macromolecule
    - Regular orthogonal lattice of point
  - Ligand ‘probe’ samples force field
  - One map for each *ligand* atom type
- AutoDock uses *trilinear interpolation*
  - to compute interaction energy between ligand and target
- Non-bonded energy is pre-calculated
- *Saves time:* ~100x faster than traditional non-bonded pair list method

AutoGrid Grid Box



- Grid box depends on:
  - Orientation with respect to protein.
  - Where should I center the grid box?
    - Center on ligand;
    - Center on macromolecule;
    - Pick atom;
    - Type in x-, y- and z-coordinates.
  - Spacing (0.2 Å - 1.0 Å: default 0.375 Å).
  - Specify an **Even** Number of x-, y-, z-points (2×2×2 - 126×126×126).
- % makebox mol.gpf > mol.gpf.box.pdb

Ligand Flexibility

- Set Root of Torsion Tree:
  - By interactively picking, *or*
  - Automatically.
    - Smallest 'largest sub-tree'.
- Interactively Pick Rotatable Bonds:
  - No 'leaves';
  - No bonds in rings;
  - Can freeze:
    - Peptide/amide/selected/all;
  - Can set the number of active torsions that move either the most or the fewest atoms

Choose the Docking Algorithm

- SA.dpf → Simulated Annealing
- GA.dpf → Genetic Algorithm
- LS.dpf → Local Search
  - Solis-Wets (SW)
  - Pseudo Solis-Wets (pSW)
- GALS.dpf → Genetic Algorithm with Local Search, *i.e.* Lamarckian GA

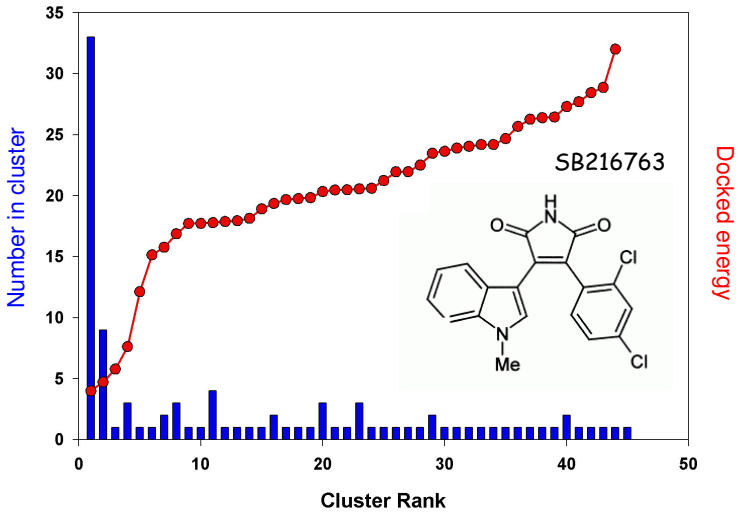
AutoDock 3 Scoring Function

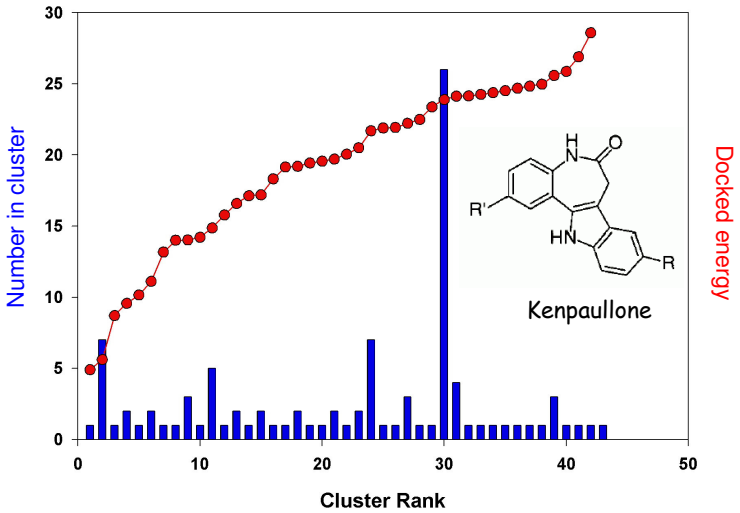
$$\Delta G_{binding} = \Delta G_{vdW} + \Delta G_{elec} + \Delta G_{hbond} + \Delta G_{desolv} + \Delta G_{tors}$$

- ΔG_{vdW}
  12-6 Lennard-Jones potential
- ΔG_{elec}
  Coulombic with Solmajer-dielectric
- ΔG_{hbond}
  12-10 Potential with Goodford Directionality
- ΔG_{desolv}
  Stouten Pairwise Atomic Solvation Parameters
- ΔG_{tors}
  Number of rotatable bonds

Viewing Conformational Clusters by RMSD

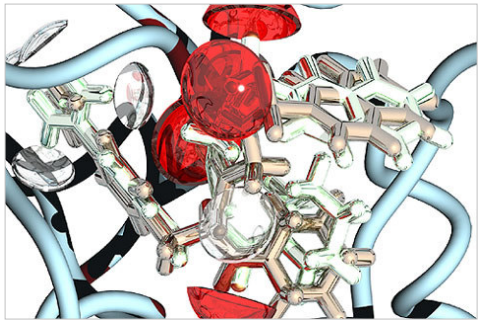
- List of available RMSD tolerances
  - Separated by spaces
- Histogram of conformational clusters
  - Number in cluster *versus* energy
- Pick a cluster
  - makes a list of the conformations in that cluster;
  - makes this the current sequence for states player.





PROGRAM **FlexX**

http://www.biosolveit.de/FlexX/



Main applications:

(1) *Binding mode prediction*

For a protein with known three-dimensional structure and a small ligand molecule, FlexX predicts the geometry of the protein-ligand complex and estimates the binding affinity in less than 15 seconds.

(2) *Virtual high-throughput screening (vHTS)*

With FlexX a database consisting of ~100.000 compounds can be screened in about 8 hours on a 30-node cluster – fully automated

Algorithmic details:

- **Incremental construction.**
- The **conformational flexibility of the ligand** is taken into account
- The **MIMUMBA database** is used for determination of low-energy torsion angles, while an interaction geometry database is used to exactly describe intermolecular interaction patterns. For scoring, FlexX uses an adapted **Böhm function**.

Program **GOLD**

- Product of a collaboration between the University of Sheffield, GlaxoSmithKline plc and CCDC
- Uses a genetic algorithm for optimization
- Can output multiple solutions (i.e. output multiple final population members)
- Full ligand and partial protein flexibility
- Fitness function combination of four elements:
  - protein-ligand hydrogen bond energy (*external H-bond*)
  - protein-ligand van der Waals (vdw) energy (*external vdw*)
  - ligand internal vdw energy (*internal vdw*)
  - ligand torsional strain energy (*internal torsion*)

Genetic Algorithms

- Create a "population" of possible solutions, encoded as "chromosomes"
- Use "fitness function" to score solutions
- Good solutions are combined together ("crossover") and altered ("mutation") to provide new solutions
- The process repeats until the population "converges" on a solution

How GAs Work

A way is found of encoding possible solutions into a bitstring (*chromosome*), and of specifying the 'goodness' of a chromosome (*fitness function*)

1. Initialize a population of chromosomes
2. Evaluate the fitness of each chromosome
3. Create new chromosomes from the current population
4. Delete population members to make room for new ones
5. Evaluate the new chromosomes and put them in population
6. If we want to keep going, go back to step 3

Genetic Algorithms

- For our purpose, we can encode rotation and translation of a molecule, and bond torsion angles in a chromosome, e.g.:

$$T_X \; T_Y \; T_Z \; R_X \; R_Y \; R_Z \; \tau_1 \; \tau_2$$

where we have 3 translation values (T), 3 rotation values (R) and as many torsion angles (τ) as the molecule has rotatable bonds

Genetic Algorithms

- Initially, our population will be initialized with random values, e.g.

	T_X	T_Y	T_Z	$R_X°$	$R_Y°$	$R_Z°$	$\tau_1°$	$\tau_2°$
C1	-3.2	-1.6	4.5	130	126	228	131	114
C2	2.8	1.3	-4.6	97	231	149	126	144
C3	-8.7	2.9	3.1	143	261	12	83	29
C4	-2.2	-2.9	-3.6	27	280	141	312	216
C5	5.8	4.1	4.9	19	25	26	341	18
C6	0.3	-2.7	5.6	14	81	27	155	75
C7	4.4	-0.3	-0.2	12	46	22	26	98

Fitness Function

- Used to score chromosomes to determine “goodness”
- For our purposes, we are concerned with how well the molecule in a particular orientation binds to the protein
- So a fitness function for a docking GA might be a combination of the following elements:
  - Energy (binding, potential)
  - Number and strength of hydrogen bonds formed
  - Hydrophobic effects
  - Electrostatic effects

Fitness Function

- To score a chromosome, the GA will place the molecule inside the protein using the given translation, rotation and torsion parameters, and the fitness function will calculate the score based on an analysis of the joint 3D structure

Fitness function scoring of population

- Initially, our population will be initialized with random values, e.g.

	T_X	T_Y	T_Z	$R_X°$	$R_Y°$	$R_Z°$	$\tau_1°$	$\tau_2°$	**Score**
C1	-3.2	-1.6	4.5	130	126	228	131	114	**0.42**
C2	2.8	1.3	-4.6	97	231	149	126	144	**0.95**
C3	-8.7	2.9	3.1	143	261	12	83	29	**0.87**
C4	-2.2	-2.9	-3.6	27	280	141	312	216	**0.04**
C5	5.8	4.1	4.9	19	25	26	341	18	**0.32**
C6	0.3	-2.7	5.6	14	81	27	155	75	**0.78**
C7	4.4	-0.3	-0.2	12	46	22	26	98	**0.61**

Create new population members

- Initially, our population will be initialized with random values, e.g.

	T_X	T_Y	T_Z	$R_X°$	$R_Y°$	$R_Z°$	$\tau_1°$	$\tau_2°$	Score
C1	-3.2	-1.6	4.5	130	126	228	131	114	**0.42**
C2	2.8	1.3	-4.6	97	231	149	126	144	**0.65**
C3	-8.7	2.9	3.1	143	261	12	83	29	**0.77**
C4	-2.2	-2.9	-3.6	27	280	141	312	216	**0.04**
C5	5.8	4.1	4.9	19	25	26	341	18	**0.32**
C6	0.3	-2.7	5.6	14	81	27	155	75	**0.78**
C7	4.4	-0.3	-0.2	12	46	22	26	98	**0.61**

Crossover

C2	2.8	1.3	-4.6	97	231	149	126	144	**0.65**
C3	-8.7	2.9	3.1	143	261	12	83	29	**0.77**

C8	-8.7	1.3	-4.6	97	261	12	83	144	

Mutation

C2	2.8	0.3	-4.6	107	221	149	126	134	**0.65**

C9	2.8	0.3	-4.6	107	221	149	126	134	

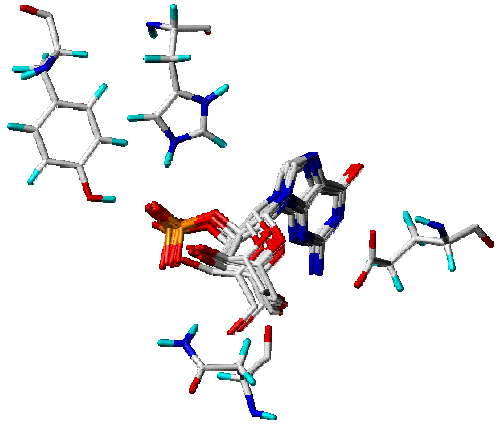
Score new chromosomes

	T_X	T_Y	T_Z	$R_X°$	$R_Y°$	$R_Z°$	$\tau_1°$	$\tau_2°$	Score
C1	-3.2	-1.6	4.5	130	126	228	131	114	**0.42**
C2	2.8	1.3	-4.6	97	231	149	126	144	**0.65**
C3	-8.7	2.9	3.1	143	261	12	83	29	**0.77**
C4	-2.2	-2.9	-3.6	27	280	141	312	216	**0.04**
C5	5.8	4.1	4.9	19	25	26	341	18	**0.32**
C6	0.3	-2.7	5.6	14	81	27	155	75	**0.78**
C7	4.4	-0.3	-0.2	12	46	22	26	98	**0.61**
C8	-8.7	1.3	-4.6	97	261	12	83	144	**0.83**
C9	2.8	0.3	-4.6	107	221	149	126	134	**0.56**

Delete poor chromosomes

	T_X	T_Y	T_Z	R_X°	R_Y°	R_Z°	τ_1°	τ_2°	Score
C1	-3.2	-1.6	4.5	130	126	228	131	114	**0.42**
C2	2.8	1.3	-4.6	97	231	149	126	144	**0.65**
C3	-8.7	2.9	3.1	143	261	12	83	29	**0.77**
~~*C4*~~	~~-2.2~~	~~-2.9~~	~~-3.6~~	~~27~~	~~260~~	~~141~~	~~312~~	~~216~~	~~**0.04**~~
~~*C5*~~	~~5.8~~	~~4.1~~	~~4.9~~	~~19~~	~~25~~	~~26~~	~~341~~	~~18~~	~~**0.32**~~
C6	0.3	-2.7	5.6	14	81	27	155	75	**0.78**
C7	4.4	-0.3	-0.2	12	46	22	26	98	**0.61**
C8	-8.7	1.3	-4.6	97	261	12	83	144	**0.83**
C9	2.8	0.3	-4.6	107	221	149	126	134	**0.56**

Sample GOLD output



GMP into RNaseT1

Program **FRED** (*OpenEye*)

- **Docking is exhaustive**
  Unlike most docking programs FRED does not use stochastic sampling to dock ligand. Rather it begins with the set of all possible orientations (to a resolution of one Angstrom, by default) of each conformer near the receptor site and selects the docked position of the ligand from this set.
- **Speed**
  FRED docks typically docks from 7 to 15 conformers per second on a single PIII-800Mhz CPU.
- **Multi-processor**
  FRED fully supports PVM (Parallel Virtual Machine) on Linux and SGI platforms. This allows FRED to take advantage of multiple processors on multiple machines while still returning a single centralized set of output.
- **Multiple scoring fuctions**
  FRED currently supports Chemscore, PLP, ScreenScore and Gaussian shape scoring. Scoring with ZAP (a PB solver written by OpenEye Scientific Software) is coming in the near future.
- **Alternative docking positions for ligands**
  FRED returns alternative docked poses for each ligand as well as the top scoring ligand.
- **Graphic preping of receptor site (with VIDA)**
  While FRED is fully functional as a command line program, the graphics program *VIDA* has a FRED wizard which can be used to set up the receptor site for Fred.

Program **GLIDE** (*Schrödinger*)

- Complete systematic search of the conformational, orientational, and positional space of the docked ligand.
  - An initial rough positioning and scoring phase that dramatically narrows the search space is followed by torsionally flexible energy optimization on an OPLS-AA nonbonded potential grid for a few hundred surviving candidate poses.
- The very best candidates are further refined via a Monte Carlo sampling of pose conformation; in some cases, this is crucial to obtaining an accurate docked pose.
- Selection of the best docked pose uses a model energy function that combines empirical and force-field-based terms.

Some important questions....

- Is there any relationship between docking and ranking accuracies?
- Will docking/scoring combinations provide better results in terms of hit rates? If so, which ones?
- Does "consensus scoring" from two or three independent scoring lists outperform single scoring?
- Will it be possible to find *a universal scoring function*?

Combined use of 3 docking algorithms (Dock, FlexX, Gold) with 7 scoring functions (Dock, FlexX, Gold, Pmf, Chemscore, Fresno, Score) for screening a 1000-compound library against two different protein targets, thymidine kinase (TK) and the ligand-binding domain of the estrogen receptor R subtype (ERR).

A specific database comprising 990 random and 10 known ligands was specifically created for each target.

Results of the virtual screening examined in terms of:

(i) docking accuracy (rmsd to known solutions),

(ii) scoring accuracy (prediction of the absolute binding free energy),

(iii) "consensus" *versus* single scoring,

(iv) discrimination of active from random compounds,

(v) hit rates and enrichment factors among the top scorers.

C. Bissantz, G. Folkers & D. Rognan - *J. Med. Chem.* **43**, 4759-4767 (2000)

Docking accuracy
[Rms deviations (non hydrogen atoms, in Å) from the X-ray pose]
(top solution of each docking tool)

	Docking method		
ligand	DOCK	FlexX	GOLD
deoxythymidine	0.82	0.78	0.72
5-iododeoxyuridine	9.33	1.03	0.77
5-iodouracil-anhydrohexitol	1.16	0.88	0.63
dhbt (not publicly available)	2.02	3.65	0.93
6-(3-hydroxy-propyl-thymine)	1.02	4.18	0.49
6-[6-hydroxymethy-5-methyl-2,4-dioxo-hexahydro-pyrimidin-5-yl-methyl]-5-methyl-*1*H-pyrimidin-2,4-dione	9.62	13.30	2.33
(North)-methanocarbathymidine	7.56	1.11	1.19
aciclovir	3.08	2.71	2.74
ganciclovir	3.01	6.07	3.11
penciclovir	4.10	5.96	3.01

Only one set of protein (TK) coordinates used: pdb code 1kim

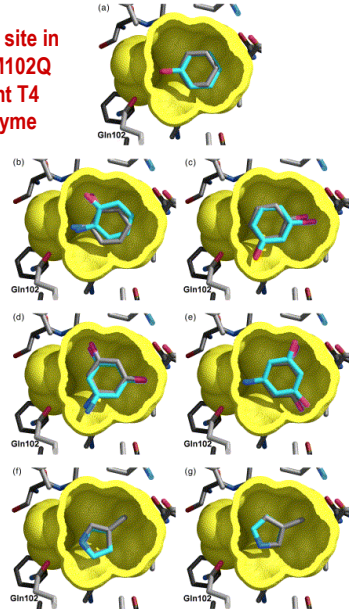
Scoring functions

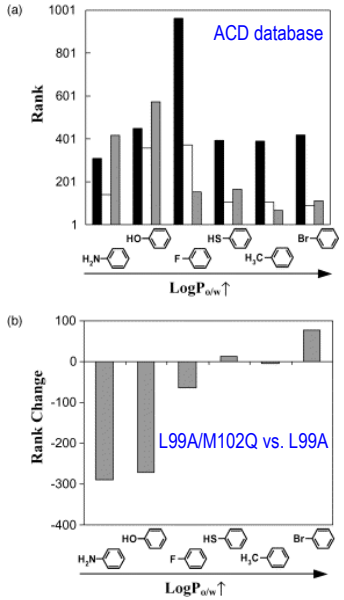
Knowledge-based: statistical analysis of 3D complex structures to derive a sum of *potentials of mean force* between receptor and ligand atoms

Force field-based: calculation of van der Waals and electrostatic interaction energies between the receptor and the ligand atoms

Empirical: the binding free energy is broken down into a number of different *weighted* contributions (supposed to be additive: number of hydrogen bonds, ionic interactions, apolar contacts, entropy penalties...)

Binding site in L99A/M102Q mutant T4 lysozyme

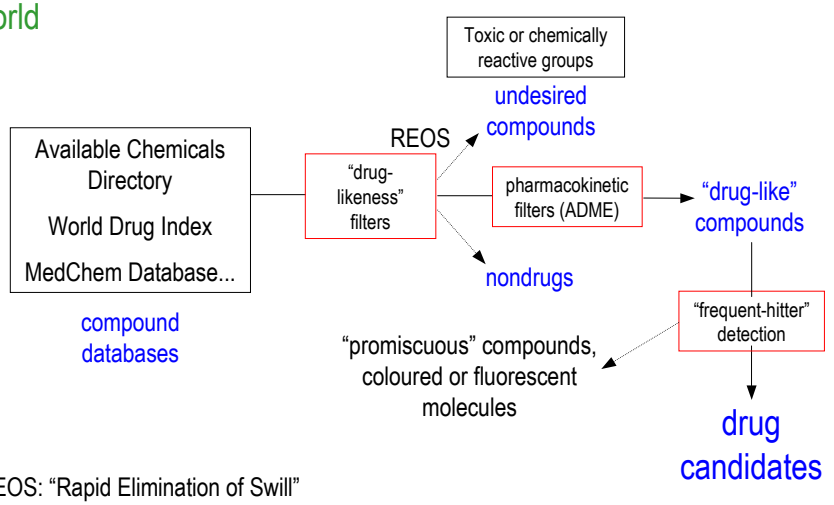




Wei BQ, Baase WA, Weaver LH, Matthews BW, Shoichet BK.
A model binding site for testing scoring functions in molecular docking.
J. Mol. Biol. (2002) 322:339-355

In silico VIRTUAL SCREENING and FOCUSED LIBRARY DESIGN

Near-perfect structures in an imperfect world

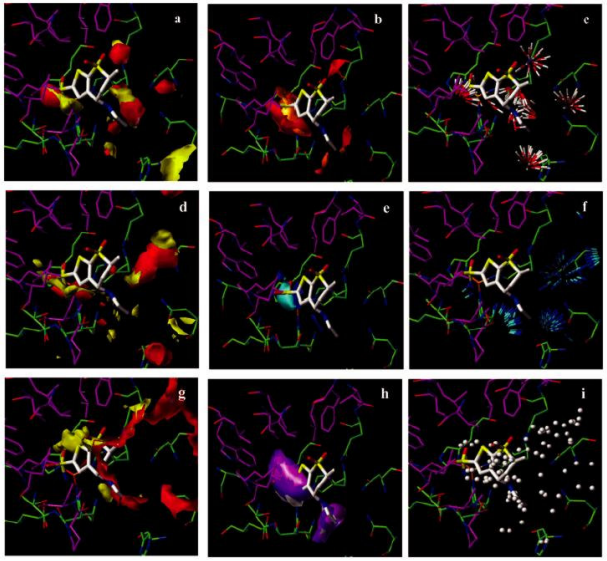


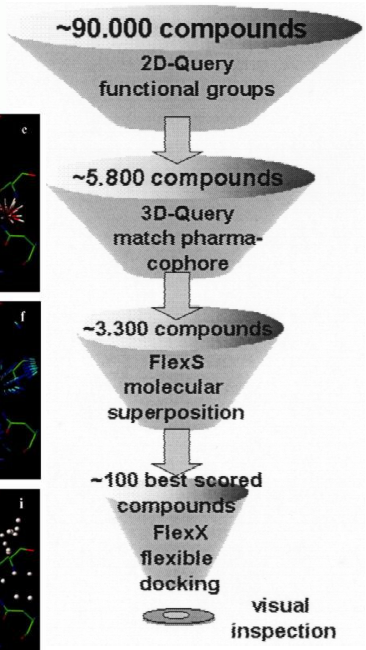
REOS: "Rapid Elimination of Swill"

3588 *J. Med. Chem.* **2002**, *45*, 3588−3602

Successful Virtual Screening for Novel Inhibitors of Human Carbonic Anhydrase: Strategy and Experimental Confirmation

Sven Grüneberg,† Milton T. Stubbs, and Gerhard Klebe*
Institute of Pharmaceutical Chemistry, University of Marburg, Marbacher Weg 6, D-35032 Marburg, Germany



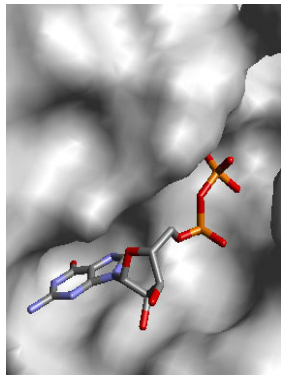


Screensaver Lifesaver

searching for anti-cancer drugs by distributed computational chemistry

http://www.chem.ox.ac.uk/ccdd/ccdd.html

NATIONAL FOUNDATION FOR CANCER RESEARCH
research for a cure



Superoxide dismutase	Vascular Endothelial Growth Factor
RAS proteins	Insulin Tyrosine Kinase
Cyclooxygenase (COX-2)	c-ABL Tyrosine Kinase
Fibroblast Growth Factor Receptor	CDK-2
RAF	Farnesyltransferase
Protein-Tyrosine-Phosphatase 1B	VEGFr1

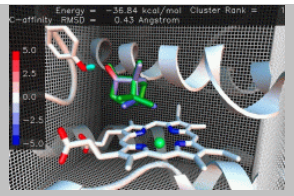
fightAIDS@home the Olson laboratory
computing toward a cure
powered by ENTROPIA

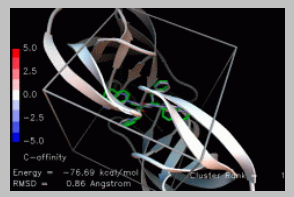
THE SCRIPPS RESEARCH INSTITUTE

http://FightAidsathome.scripps.edu

Dr. Garrett Morris

fight AIDS @home
computing toward a better inhibitor
powered by AUTODOCK





Applications of Ligand-Protein Docking in Drug Design

Existing methods

Given a protein, find potential binding ligands from a chemical database

Compound Database
Compound 1
...
Compound n

↓ Protein

↓ Successfully docked compounds as putative ligands

***Science* (1992) *257*: 1078**

New method

Given a ligand, find potential potein targets from a protein database

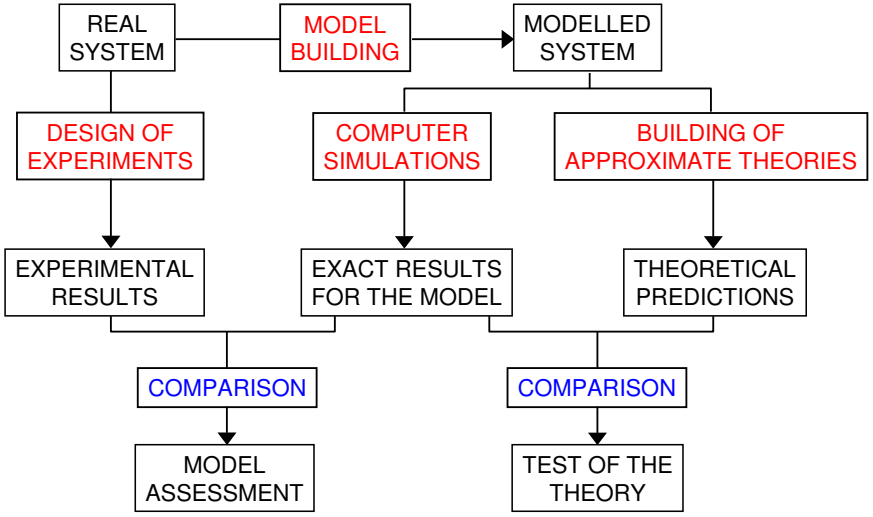
Protein Database
Protein 1
...
Protein n

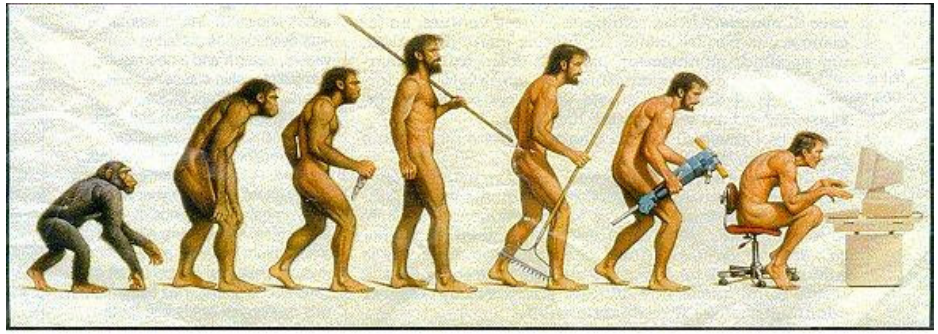
↓ Ligand

↓ Successfully docked proteins as putative targets

***Proteins* (2001) *43*: 217**

CONNECTION BETWEEN EXPERIMENT, THEORY AND COMPUTER SIMULATION





Somewhere, something went terribly wrong

QUESTIONS WELCOME

PREGUNTAS, POR FAVOR

E-mail: federico.gago@uah.es