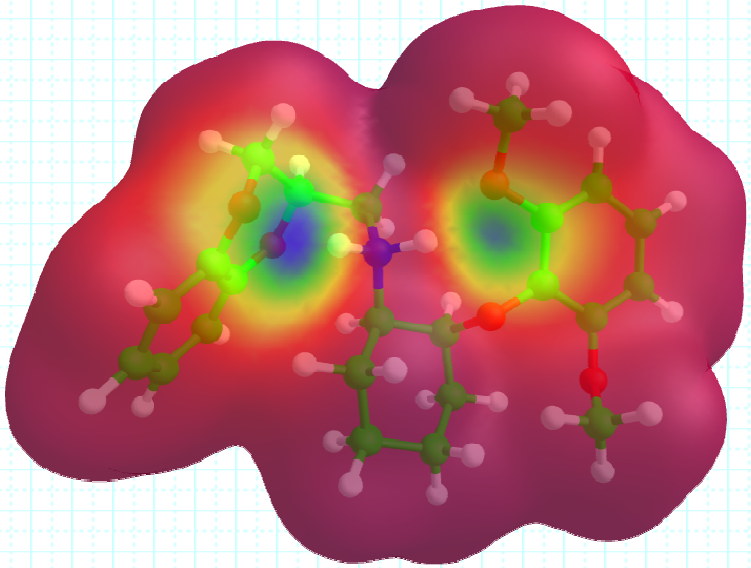
Università degli Studi di Milano
Dipartimento di Scienze Farmaceutiche "Pietro Pratesi"

Workshop on VEGA ZZ

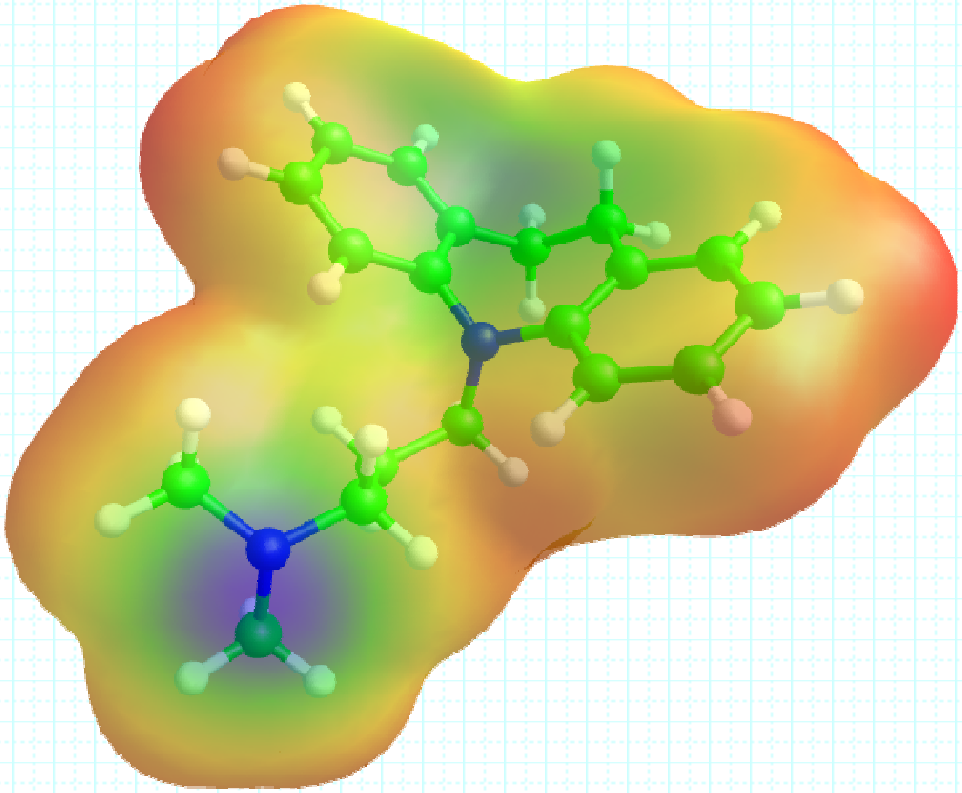
Practical session

Belgrade, March 22, 2009

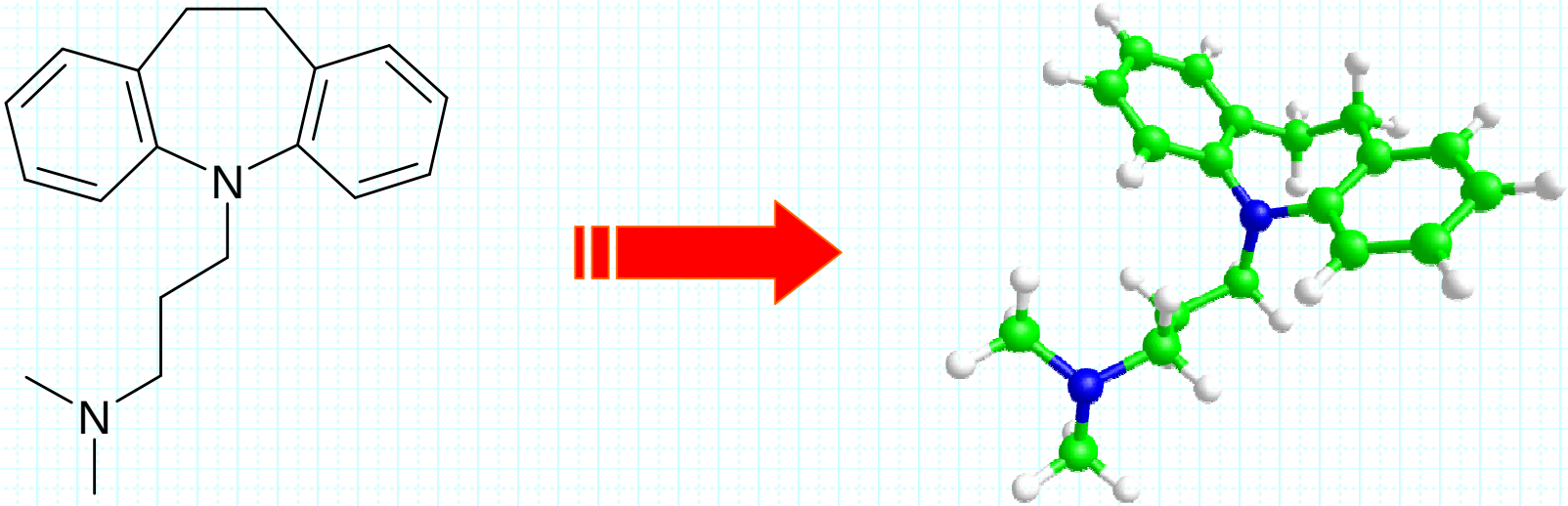


Alessandro Pedretti

How to analyze the conformational properties of a small molecule



How to build imipramine

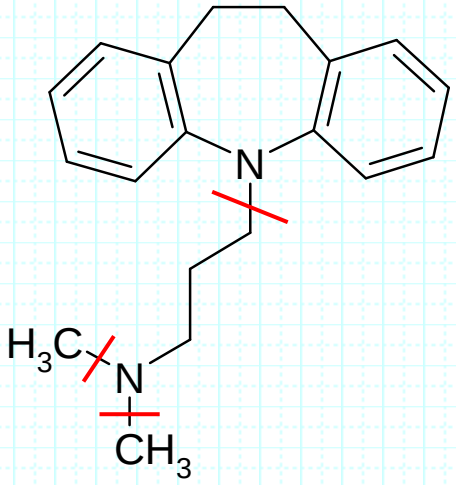


You can build molecules by two different approaches:

- Built-in 3D molecular editor.
- 2D molecular editor (e.g. ISIS/Draw 2.5, JME, JChemPaint, Sketchel etc.)

3D molecular editor

The 3D editor is based on fragments databases containing building blocks that can be combined each other to complete a more complex structure. For this reason, you must fragment the molecule in less complex fragments that will be assembled as indicated in the following scheme:

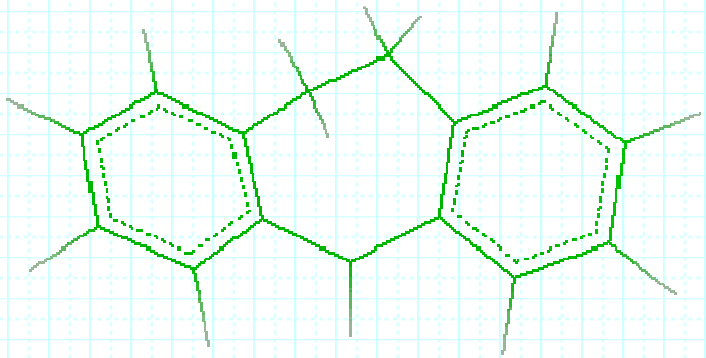


Excluding initially the heteroatoms, the system can be fragmented in:

- #1 tricyclic system (Dibenzo[a,d]cycloheptane);
- #1 n-butyl chain;
- #2 methyl groups.

Building the molecule[1]

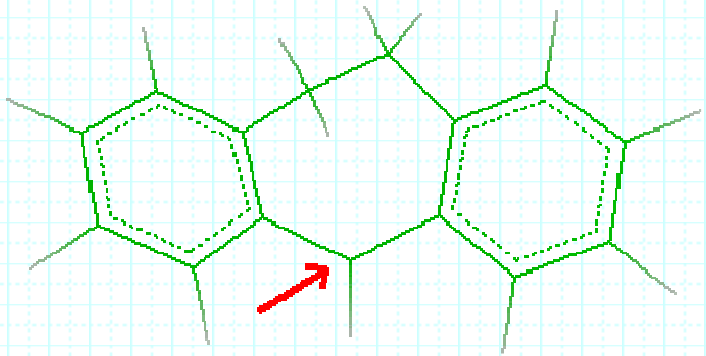
- Select *Edit → Add → Fragments* in the menu bar and the *Add fragment/s* window will appear.
- Choose *Rings (aromatic)* in the *Group* box and *15 5H-Dibenzo[a,d]cycloheptane* in the *Fragment* box.
- Click the *Finish* and thus *Close* buttons. The starting building block is shown in the workspace:



15 5H-Dibenzo[a,d]cycloheptane fragment

Building the molecule[2]

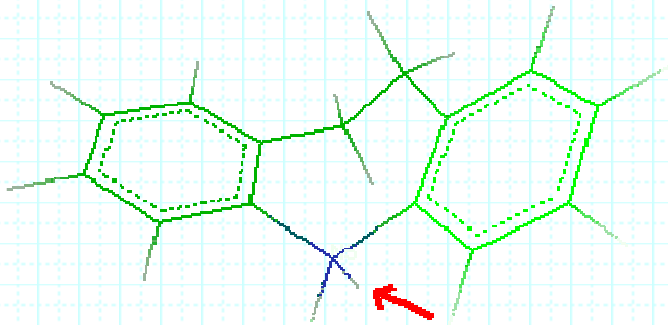
- In order to change the *C5* carbon to a nitrogen and to remove one hydrogen connected to it, select *Edit* → *Change* → *Atom/residue/chain* in the menu bar.
- Click the *C5* as indicated by the red arrow in the following picture:



- In the *Element* field of the *Edit* dialog, change *C* to *N*, click the *Apply* button and close the window. The atom color will change from green to blue.

Building the molecule[3]

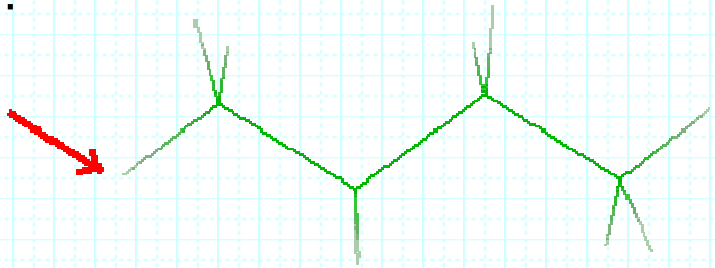
- Rotating the molecule, highlight the two hydrogens bonded to *N5*.
- To remove one hydrogen, select *Edit* → *Remove* → *Atom* and click the atom to remove as indicated by the red arrow:



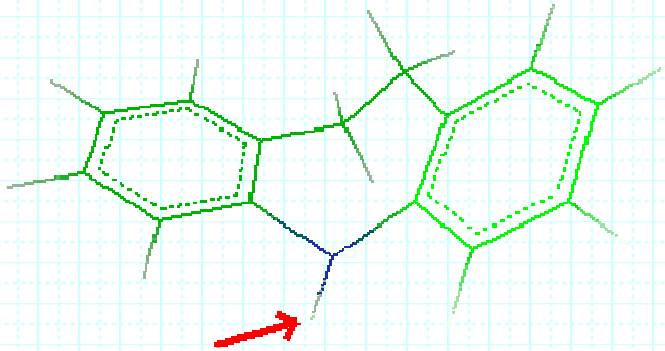
The hydrogen will be deleted. Click the *Done* button to close the window.

Building the molecule[4]

- To add the n-butyl chain, reopen the *Add fragment/s* window (*Edit* → *Add* → *Fragments*), search *04C n-Butane* in the *Alkanes* group and click the *Next* button.
- Click the butane hydrogen that will be merged with the hydrogen bonded to *N5* (see red arrow):

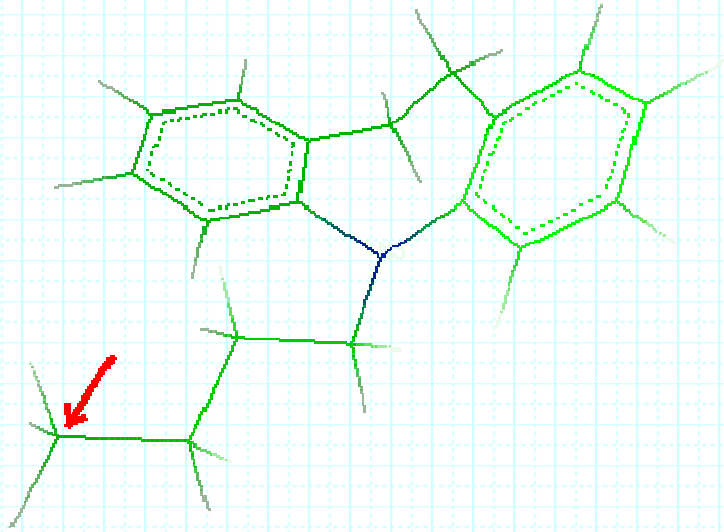


- Click the *Next* button and the tricyclic system will be shown. Click the hydrogen bonded to *N5*:



Building the molecule[5]

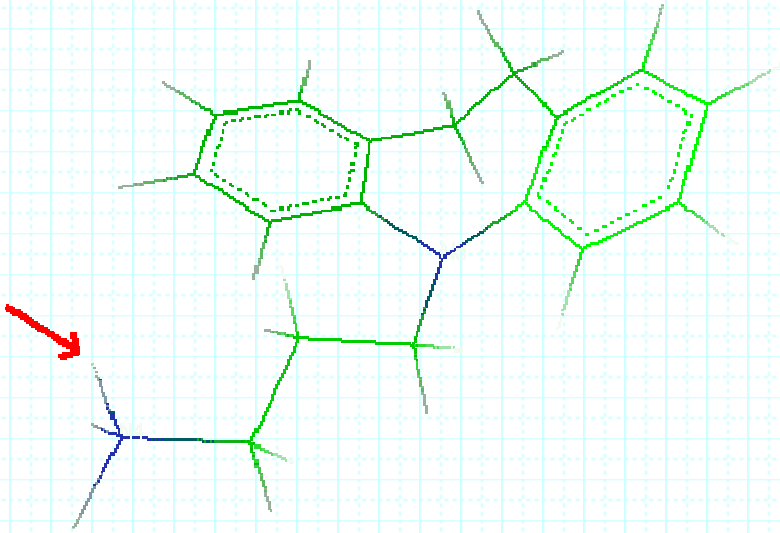
- Click *Next* and thus the *Finish* buttons. Close the window.
- At this step, you must change the *C4* of the n-butyl chain to a nitrogen and remove a hydrogen: select *Edit* → *Change* → *Atom/residue/chain* and click the *C4* as indicated by the red arrow:



- In the *Element* field of the *Edit* dialog, change *C* to *N*, click the *Apply* button and close the window.

Building the molecule[6]

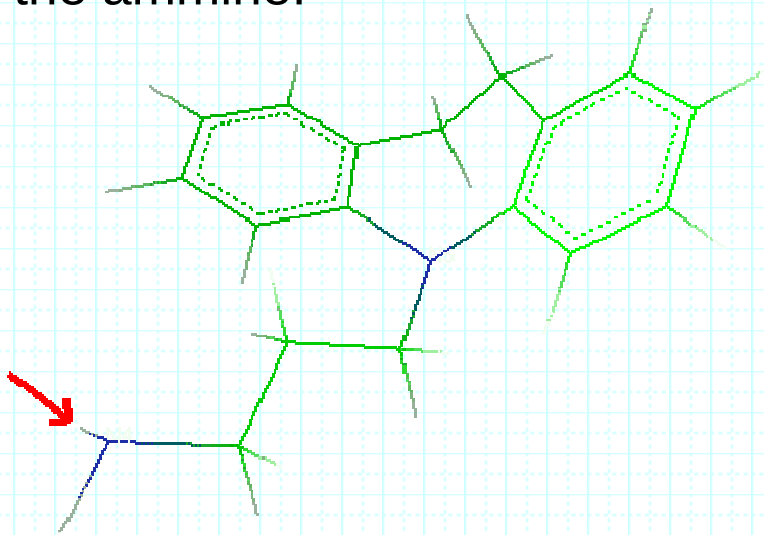
- To remove one hydrogen, select *Edit* → *Remove* → *Atom* and click the atom to remove:



- Click the *Done* button to close the window.
- Finally you must add the two methyl groups to the *N4* of the n-butyl chain. Open the *Add fragment/s* window (*Edit* → *Add* → *Fragments*).

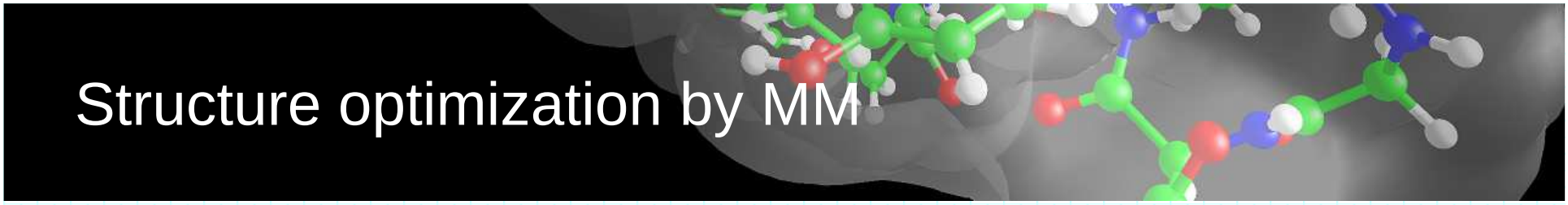
Building the molecule[7]

- In the *Alkanes* group select *01C Methane* and click *Next*.
- Click a methane hydrogen and the *Next* button.
- Click a hydrogen of the ammine:



- Click *Next* and *Finish*.
- Repeat the same steps to add the second methyl group:
- Save the molecule in IFF format (*File → Save As...*) with the *imipramine.iff* file name.

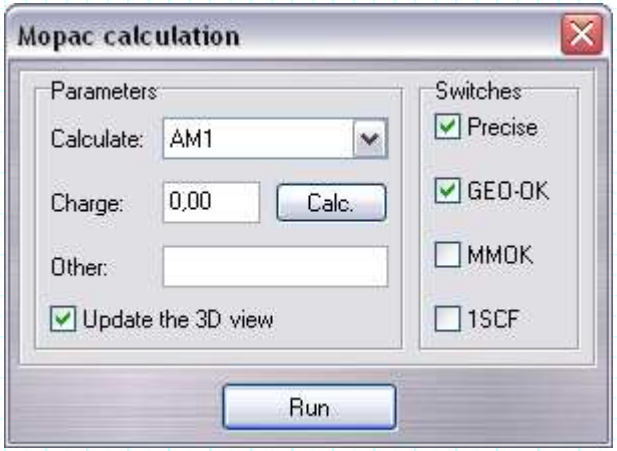
Structure optimization by MM



- Assign the atom types and the charges (*Calculate → Charge & Pot.*), checking *Force field* and *Charges* and selecting *SP4* and *Gasteiger*. Click the *Fix* button. The total charge is 0.
- Open the *Ammp minimization* window selecting *Calculate -> Ammp -> Minimization* in the menu bar.
- Choose *Conjugate gradients* and set *Minimization* steps to *1000* and *Toler* to *0.01*.
- Click the *Run* button. After few steps, the minimization is completed.
- Save the molecule in *IFF format* overwriting the previous one.

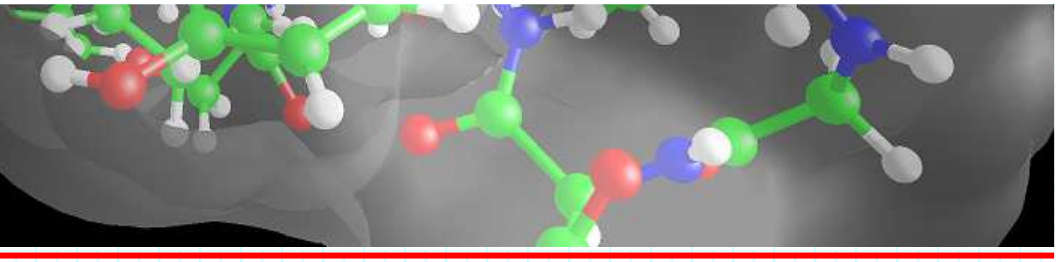
Semi-empirical calculation with Mopac

- Open the *Mopac calculation* window, selecting *Calculate → Mopac* in the main menu.
- In the *Parameters* box, select *AM1* in the *Calculate* field, keep checked the *Precise* and *GEO-OK* items.



- Clicking the *Run* button, the file requester is shown. Keep the default file name and click the *Save* button to start the calculation. The graphic will be updated during the calculation only if Mopac 2007-2009 is installed.
- Normalize the atom coordinates (*Edit → Coordinates → Normalize*).
- Save the molecule in *IFF format* overwriting the previous one.

Conformational search[1]



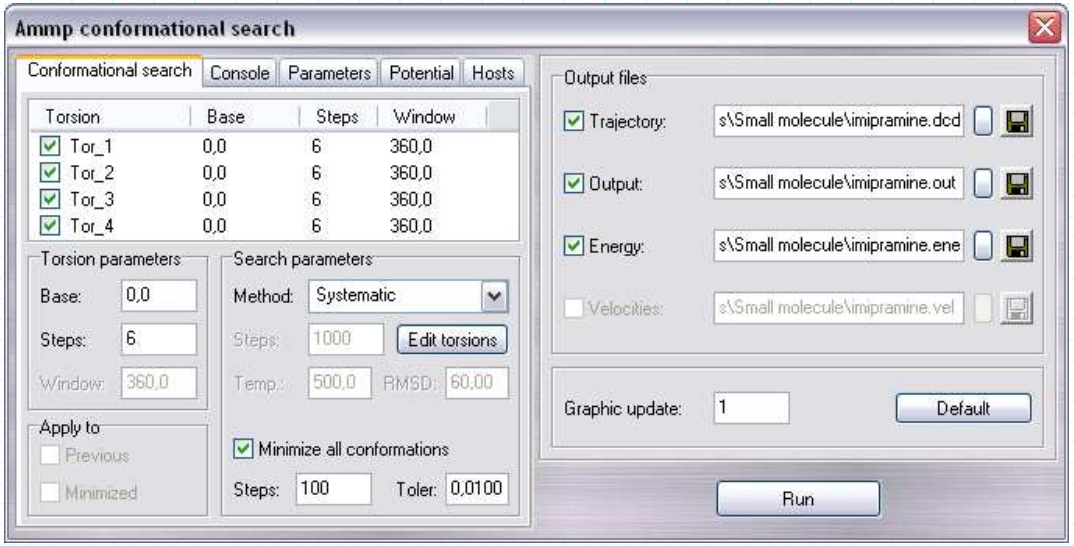
In order to find a reasonable lowest energy conformation, a conformational search will be performed. The flexible torsions (dihedrals) will be systematically rotated by an angle value (*grid scan*) and each conformation will be optimized in order to find the best minimum.

- Open the *Ammp conformational search* window (*Calculate* → *Ammp* → *Conformational* search).
- Click the *Edit torsion* button. The *Selection tool* window will appear.
- In its menu bar, select *Edit* → *Add flexible torsions* and all flexible torsion will be automatically added in the selection list (4 torsions). They are highlighted in the workspace also.
- Click *Done* and the *Ammp conformational search* window is shown.
- Select *Systematic* in the *Method* field of the *Search parameters* box.

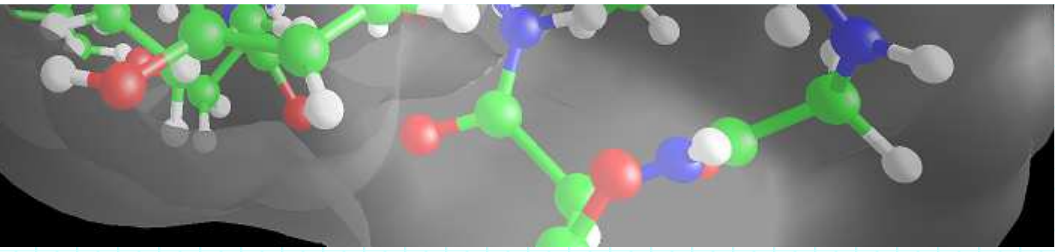
Conformational search[2]

The upper box shows the torsions that will be rotated during the scan. Increasing the number of rotation steps (see the *Steps* field in the *Torsion parameters* box), the search is more accurate but more computational time is required. The value of 6 is a good choice because it means a rotation of 60 degrees for each step.

- Check *Trajectory*, *Output* and *Energy* in the left box. Please remember to set the *Graphic update* to *1*, otherwise not all conformations will be stored in the output files.
- Check *Minimize all conformations*.
- Put *100* in the *Steps* field and *0.01* in the *Toler* field.



Conformational search[3]

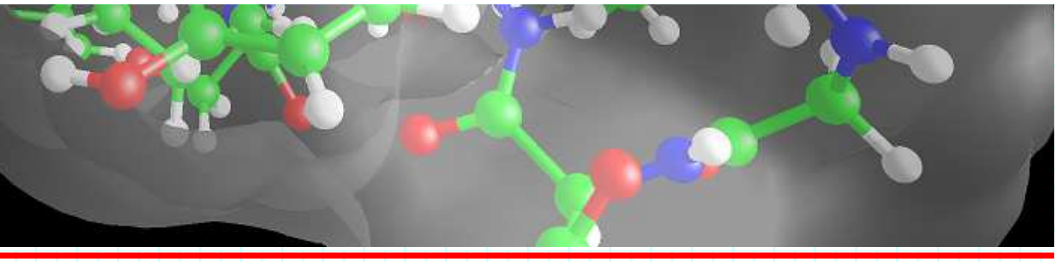


- Click the *Run* button to start the calculation. In the console will be shown for each conformation the starting energy, the best energy found at that time, and the energy after the minimization.
- After few minutes, the search is finish and the best conformation is automatically loaded in the workspace.
- To refine this structure, repeat the energy minimization as explained above and save the final molecule.

WARNING:

Remember to uncheck the *Trajectory*, *Output* and *Energy* fields, otherwise the conformational search outputs will be lost.

Cluster analysis



You need to perform a cluster analysis of the conformations generated by the conformational search because, due to the energy minimization, some of them are similar (= they are the same energy minimum). The cluster analysis allows to discard similar conformation that are redundant for other analyses.

- Open the file *imipramine.dcd* (*File → Open*) and click *Replace* in the *Molecule placing* window. The *Trajectory analysis* window will be shown.
- Choose the *Cluster* tab. In the *Method* box, select *Torsion RMSD* and put *60* (degrees) in the *RMSD* field. It means that torsions that differs less than 60 degrees are considered similar.
- Click the *Edit torsion* button and select the flexible torsions as explained for the conformational search.
- Click *Ok* and save the clustered trajectory with the default name (*imipramine_clust.dcd*) and the default file format (*DCD*).
- The number of unique conformations is 55 (the starting number was 1296).

Analysis of the property space[1]

Some molecular properties are related to the conformations and can influence biological effects (activity, metabolism, etc). Now we calculate the Polar Surface Area (PSA) for each conformation in the cluster analysis trajectory. The PSA is considered a measure of the capability to do hydrogen bonds.

- Open the *Trajectory analysis* window (*Calculate → Analysis*) and open the file *imipramine_clust.dcd*, clicking the envelope button or dragging & dropping the file over the *Trajectory analysis* window.
- Choose the *Calculation* tab and in the *Property* field, select *Polar surface area (PSA)*.
- Click the *Ok* button and look the output in the VEGA ZZ console:

```
Starting PSA          : 1.4 Å²
Range                 : 0.0000 (3) <-> 7.4256 (8)
Average value         : 3.0439 ±2.7524
```

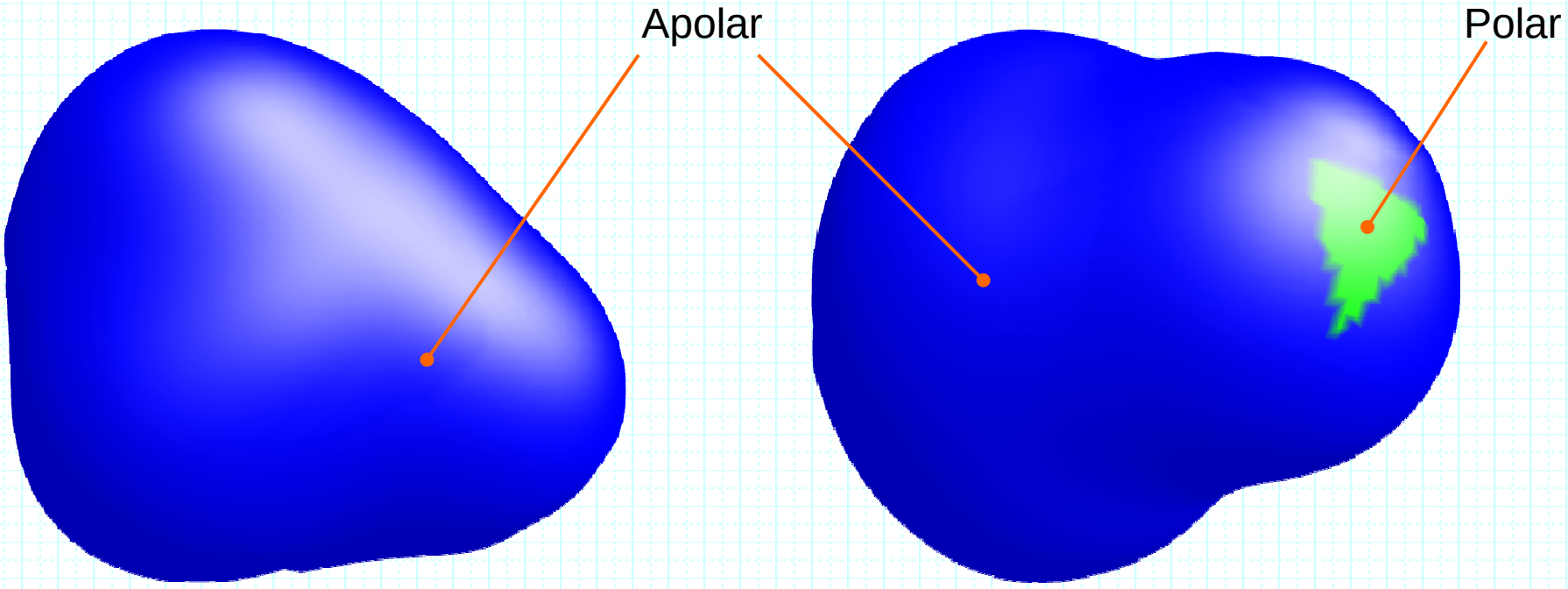
It means that the conformation *3* don't have any accessible polar atom and the conformation *8* has the maximum accessibility of polar atoms.

Analysis of the property space[2]

These results can be visualized calculating the PSA for both conformations.

- Close the Graph and reopen the *Trajectory analysis* window.
- Choose the *Selection* tab and in the *Frame number* field, put *3*. The conformation 3 will be automatically selected.
- In the main menu, choose *Calculate → Surface* and the *Surface manager* windows will be shown.
- In the *New* tab, select *Solid*, *PSA* in the *Type* field and check *Color by gradient*.
- Click the *Calculate* button and the PSA surface will be calculated. It appears completely blue and it means that is totally apolar.
- Remove the surface, clicking *Remove all* in the *Surface management* window and repeat the same steps for the conformation *8*.

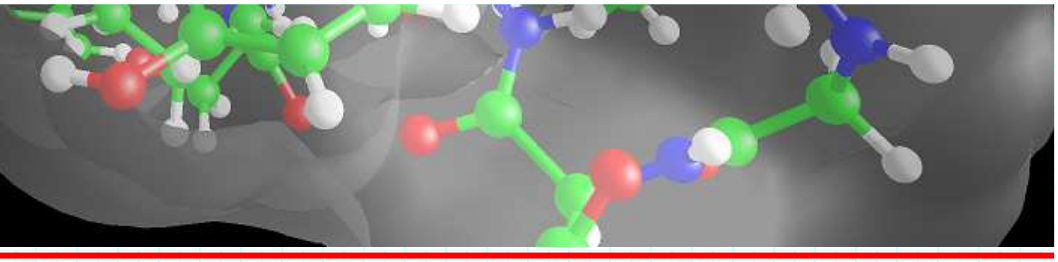
Analysis of the property space[2]



Conformation 3 Conformation 8

- Repeat the same calculation for the *Molecular lypophilicity (MLP)*.

Molecular dynamics[1]

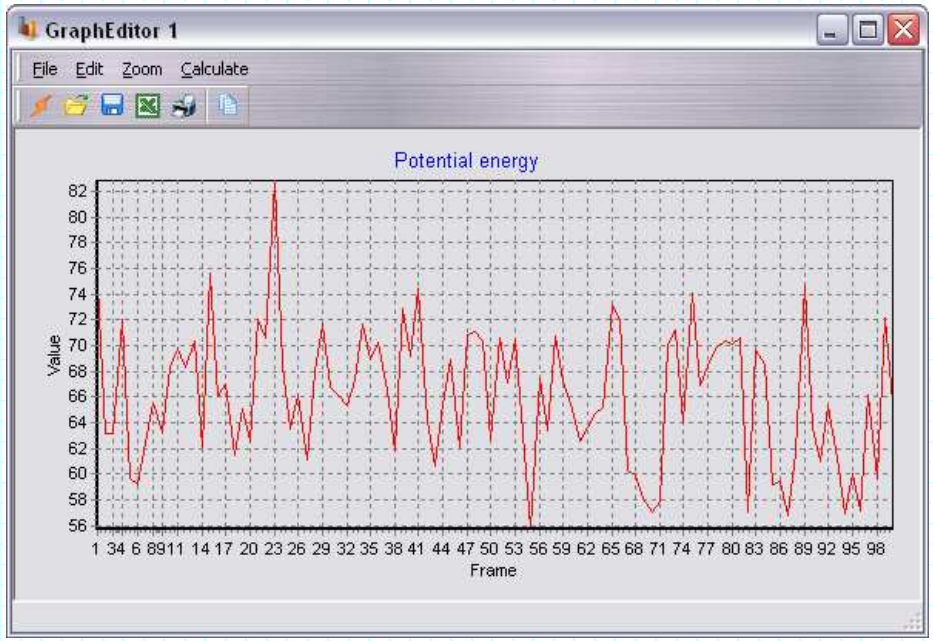


The molecular dynamics (MD) is another approach to do the conformational space analysis.

- Open the *imipramine.iff* molecule replacing the content of the current workspace.
- Assign the *CHARMM* atom types (*Calculate → Charge & Pot.*), checking *Force field*, unchecking *Charges* and selecting *CHARMM*. Click the *Fix* button.
- Save the molecule as *imipramine_dyn.iff* in IFF format (*File → Save As…*)
- Open the *NAMD dialog* window (*Calculate → NAMD*), select the *Other* tab and in the *Presettings* box, double click on *Dyn – 100 ps langevin 300K* to load the settings required to perform a MD of 100 ps in a thermostatic bath at temperature of 300 K.
- Click the *Run* button and the *Missing parameter table* is shown because three parameters aren't included in the force field.
- In the menu of that window, select *Edit → Auto assign* and click *Ok*.

Analysis of the molecular dynamics results[1]

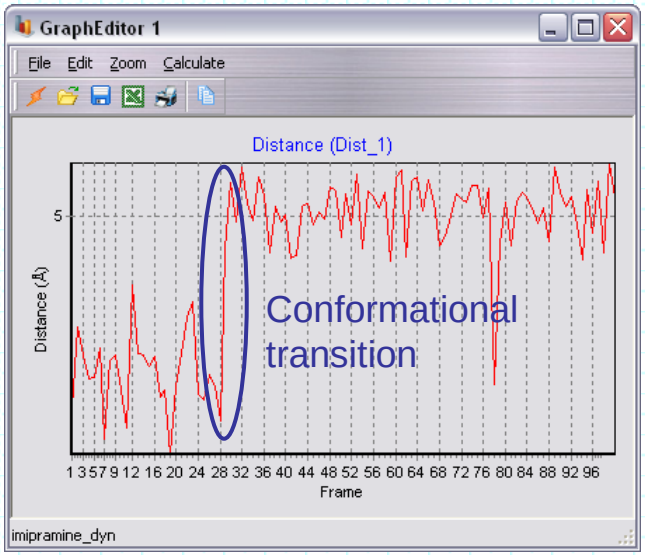
- Wait the end of the calculation.
- Open the *imipramine_dyn.dcd* file (*File → Open*).
- In the *Selection* tab, click the *Energy Graph* button to watch the potential energy plot:



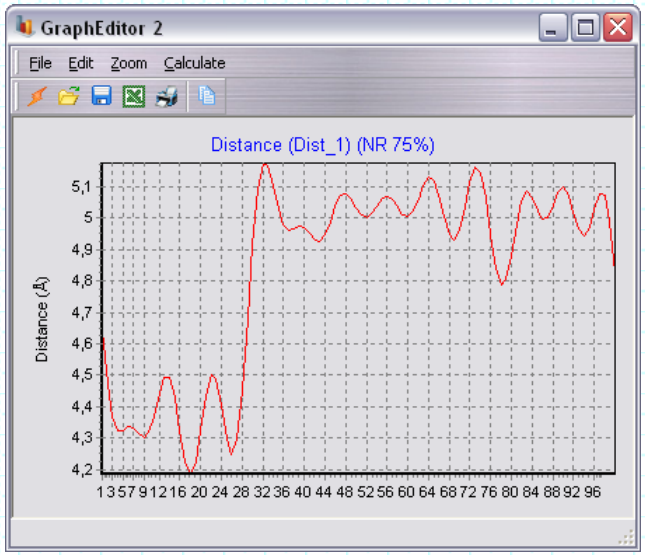
- This plot seems to not contain interesting information.

Analysis of the molecular dynamics results[2]

- In the *Trajectory analysis* window, choose the *Measure* tab, and click the *Edit* button. The *Selection tool* will be shown.
- Choose *Distance* in the selections field and click the *Add* button.
- In the main window, click on both nitrogens and finally click *Done* in the *Selection tool*.
- In *Trajectory analysis*, click the *Ok* button and the following graph is shown:



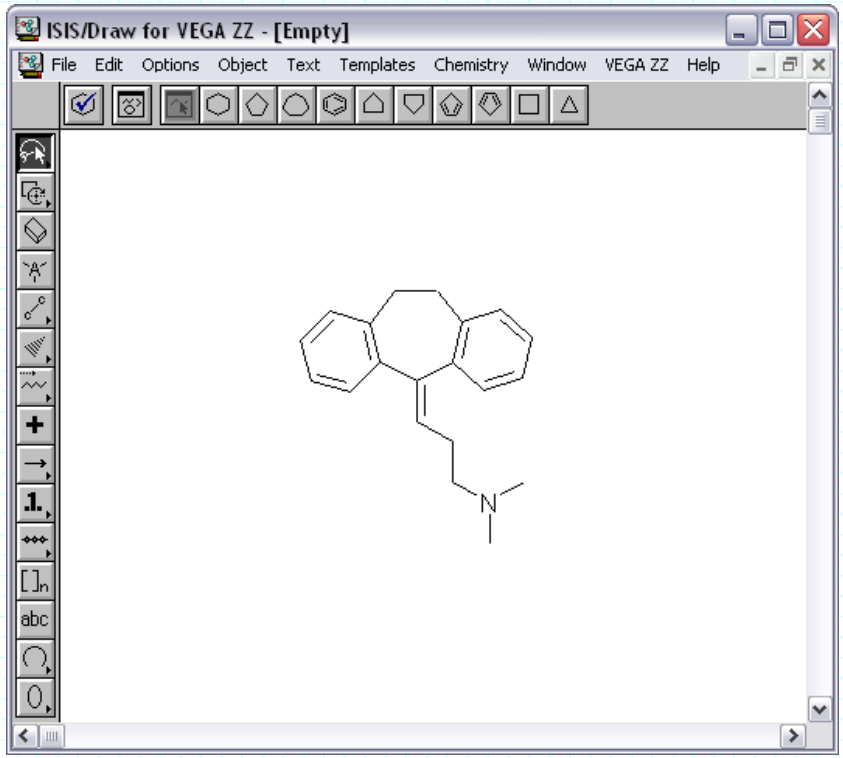
Calculate → Noise reduction → 75 %



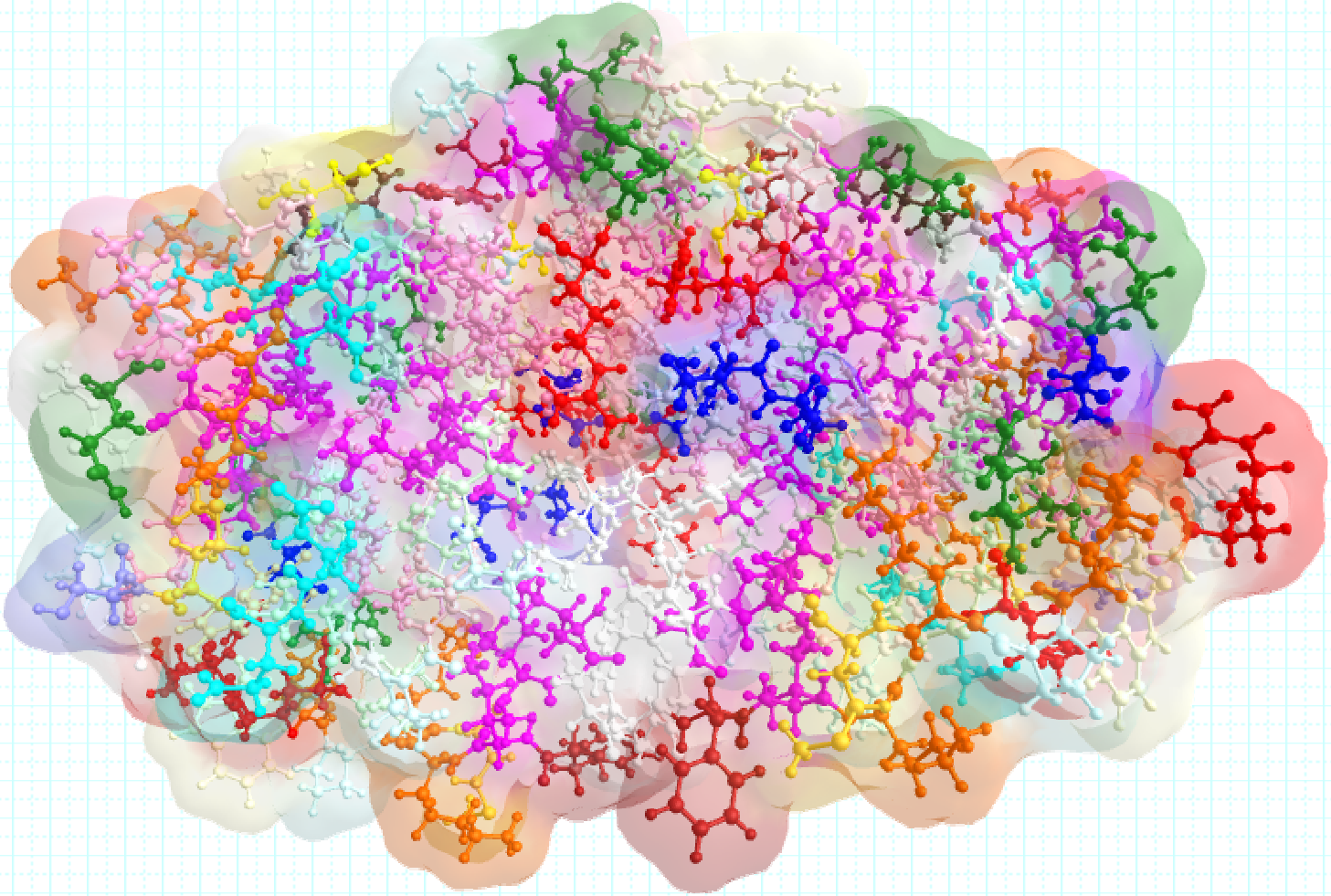
2D editing

To build a small molecule as *amitriptyline*, you can use a 2D molecular editor as ISIS/Draw 2.5 that must be installed on your PC to use it inside VEGA ZZ.

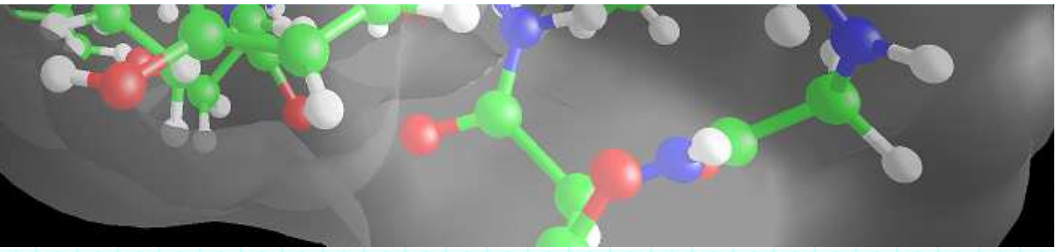
- Make sure that the workspace is empty. If isn't true, select *File → New* in the main menu.
- Open ISIS/Draw, selecting *Edit → ISIS/Draw*.
- In the ISIS/Draw environment, edit the amitriptyline.
- To transfer the molecule to VEGA ZZ, choose *VEGA ZZ → Send to VEGA* in the ISIS/Draw menu. The 2D structure will be automatically converted to 3D.
- Repeat all steps as for *imipramine*.



Virtual screening with VEGA ZZ and GriDock



What you need



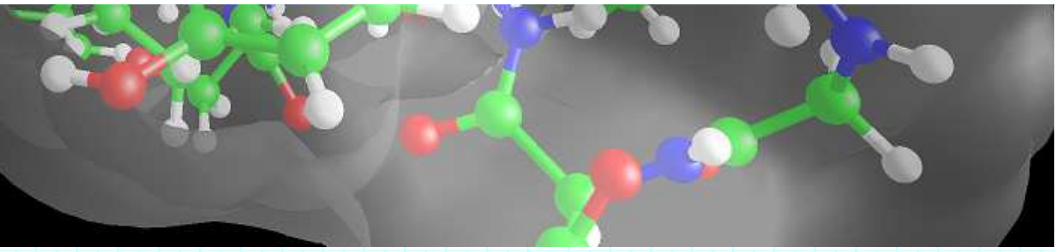
- VEGA ZZ release 2.3.0 or greater.
- NAMD for Windows (click here to download it).
- GriDock 1.0.0 virtual screening software for Windows.
- AutoDock 4 and AutoGrid 4 molecular docking package.
- Accelrys CHARMM 22 parameter files (PARM.PRM).
- Test protein. In this tutorial will be used the crystallographic structure of the HIV-1 protease complexed with VX-478, a potent inhibitor (*1HPV*) available at Protein Data Bank (*www.rcsb.org*).
- One or more databases of 3D molecules in SDF or Zip format. They can be downloaded for free at *http://ligand.info*.

Download of the target protein

Download the HIV-1 protease structure (*1HPV*) through the PDB Web interface or the tool integrated in VEGA ZZ:

- Start VEGA ZZ and select the *File → PDB* download menu item.
- Put *1HPV* in the *PDB Id* field and click *Download*. At the download end, the protein structure will be shown in the workspace.
- Normalize the coordinates in order to translate the protein at the origin of the Cartesian axis (*Edit → Coordinates → Normalize*).
- Save the molecule (*File → Save As*) with the *1HPV_orig* file name. It's strongly recommended the use of the IFF/RIFF file format because it's able to keep the maximum number of information (e.g. atom types, charges, bond orders, etc).

Protein preparation[1]

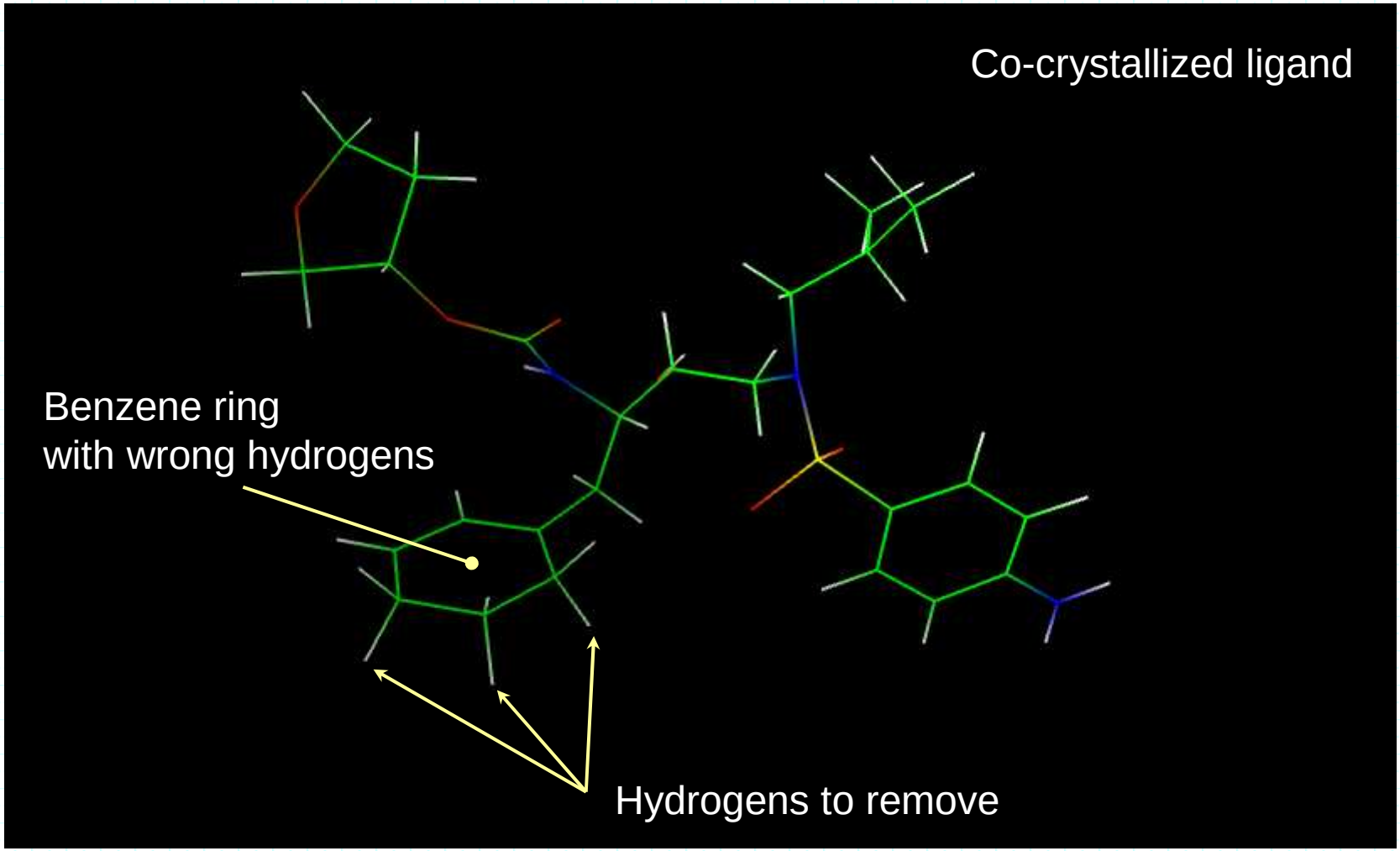


- Add the hydrogens (*Edit* → *Add* → *Hydrogens*), selecting *Protein* in the *Molecule type* box to enable an extra check for the atom hybridization, *Residue* end in the *Position of hydrogens* box and checking *Use IUPAC atom nomenclature*. Finally, click *Add* to place the hydrogens.

It may be possible that the hydrogens are incorrectly added to the co-crystallized ligand due to the protein-specific algorithm and the unusual geometry that the ligand could assume in the binding pocket.

- Show the *Atom selection* window (*View* → *Select* → *Custom*), select 478 in the *Residue* column and click the **+** button. Four carbons in one benzene ring have a wrong valence and four hydrogen atoms must be removed.
- Select *Edit* → *Remove* → *Atom* and click on the hydrogens to remove and close the dialog window pressing the *Done* button.

Protein preparation[2]

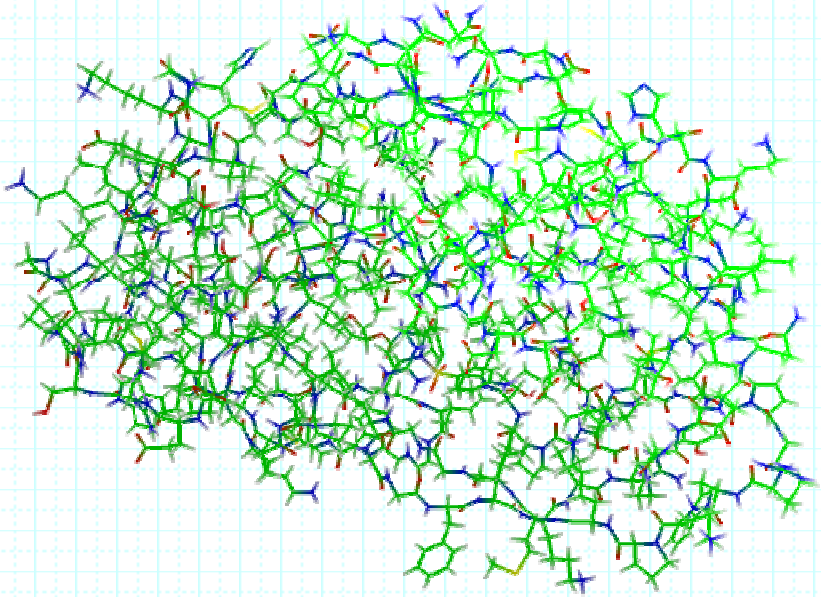


Protein preparation[3]

To optimize the structure that we completed adding the hydrogens, atomic charges and the atom potentials must be assigned.

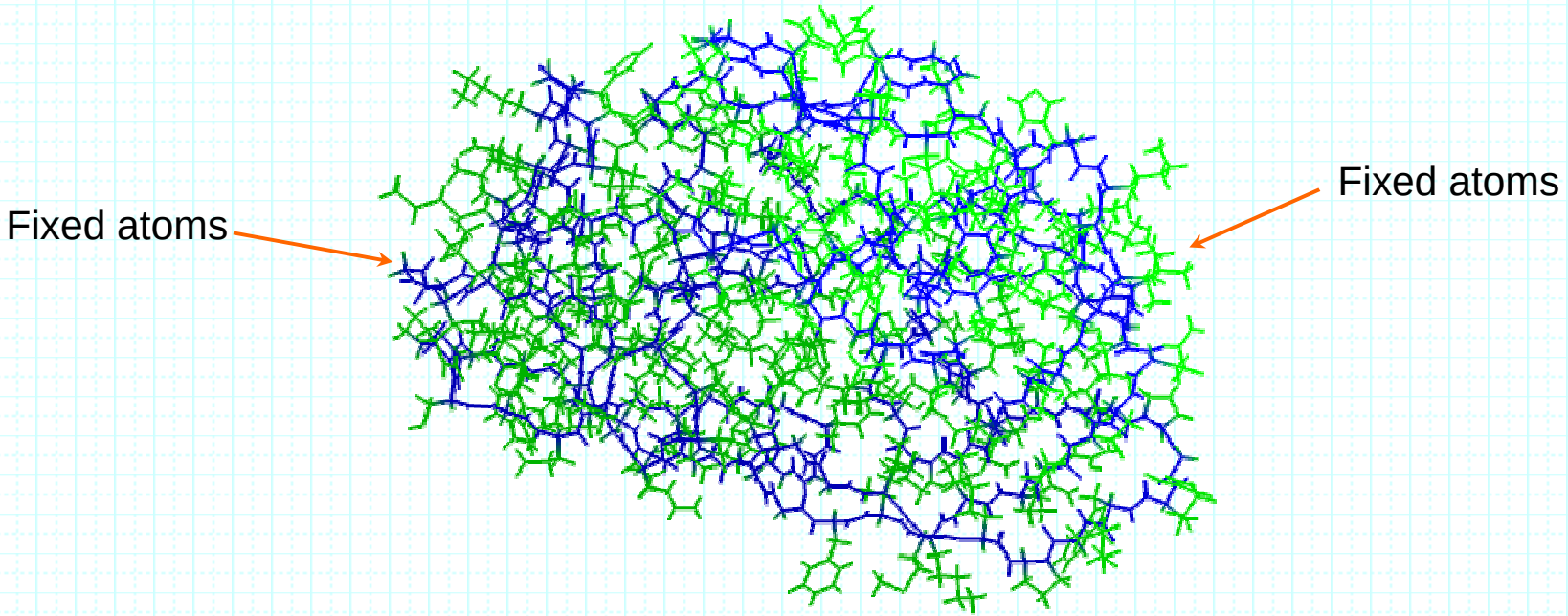
- Fix atom types and charges (*Calculate → Charge & Pot.*), checking *Force field* and *Charges* and selecting *CHARMM* and *Gasteiger*. Click the *Fix* button. The total charge is 4.
- Save the molecule in IFF format as *1HPV.iff*.

Now we are ready to run the NAMD minimization, but in order to preserve the starting experimental structure, we need to constraint the protein backbone which coordinates will be kept fixed during the calculation.

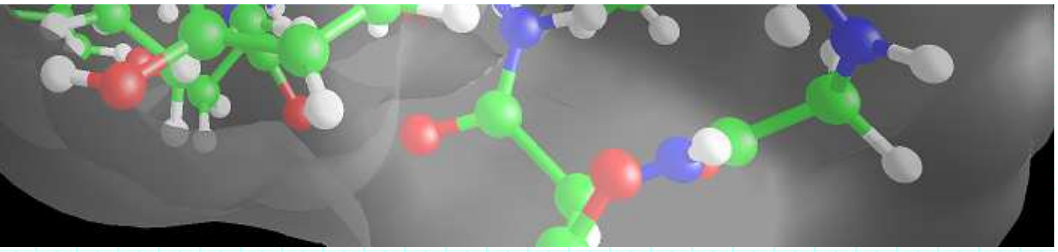


Protein minimization[1]

- Select all atoms (*VIew* → *Select* → *All*).
- In the main menu, choose *Edit* → *Coordinates* → *Constraints* and select *Fix* in the *Mode* box and *Protein backbone* in the *Selection* box. Finally click the *Apply* button. The fixed atoms (the backbone) will be colored in blue and the free atoms in green. Close the *Constraint options* window.

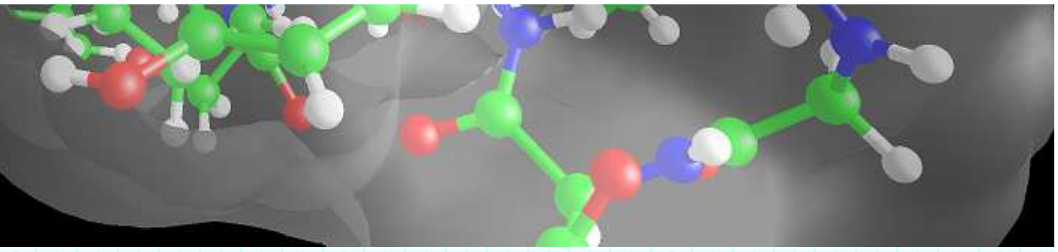


Protein minimization[2]



- Open the *NAMD dialog* window (*Calculate → NAMD*), select the *Other* tab and in the *Presettings* box, double click on *Min - Atom fixed* to load the settings required to perform an energy minimization with atom fixing.
- Go to the *Basic* tab and set the *Number of timesteps* in the *Timestep parameters* box to *10,000*.
- Clicking the *Run* button, the Missing parameter table is shown, because three ligand improper angles aren't included in the force field parameters. Click the *Ok* button without to fix the problem because the ligand will be removed from the binding pocket and thus its geometry isn't interesting for the screening.
- At the end of the calculation, save the refined structure as *1HPV_min.iff*.

Input files for GriDock[1]



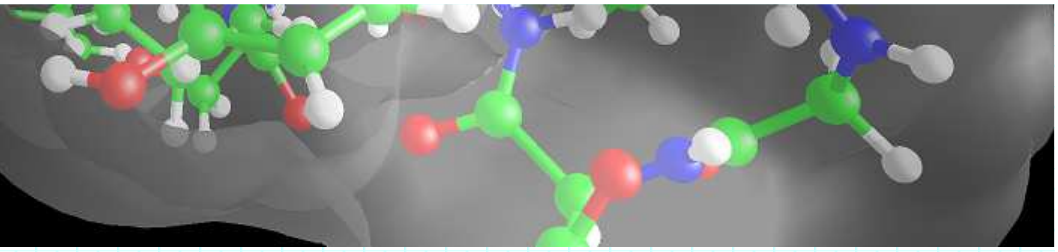
The crystallization water molecules aren't need and can create problems because they are considered fixed and can't be moved by the ligand during the docking calculation.

- To remove the water, select *Edit* → *Remove* → *Water* in the main menu.

In order to generate complexes in which the ligand is placed in the same pocket of the co-crystallized one, you need to select the atoms included in a sphere of 10 Å radius centered on the binding site.

- Find all molecules in the complex, selecting *Edit* → *Molecules* → *Fix*. Three molecules will be found.
- Select the ligand (*View* → *Select* → *Molecule*), choosing the third molecule and clicking the *Select* button. The co-crystallized ligand will be shown.
- Open the *Atom selection* window (*View* → *Select* → *Custom*) and choose the *Proximity* tab. In the *What* box, select *Atoms*, in the *Around* box, select *Molecule* and click on one atom of the molecule in the workspace. Put *10* in the *Radius* field and finally click the **+** button.

Input files for GriDock[2]

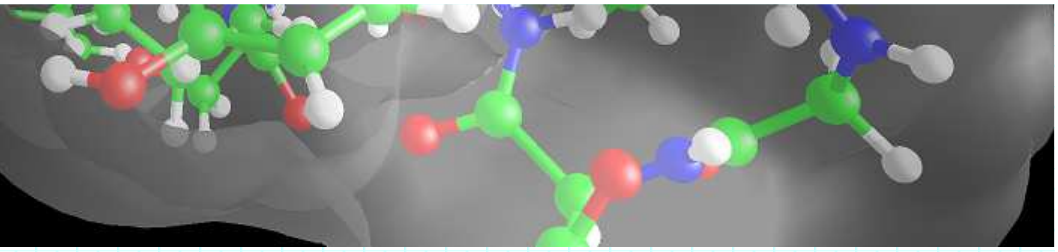


- Remove the ligand to create enough space to dock the new molecules: choose *Edit → Remove → Molecule*, select the third molecule and click the *Remove* button.

To run virtual screenings with GriDock, the receptor structure must be pre-processed assigning the AMBER atom types, fixing the atom charges, removing the apolar hydrogens and saving the molecule in PDBQT format. All these steps are automatically performed by *AutoDock\Receptor.c* script.

- Run the AutoDock\Receptor.c script (*File → Run script → AutoDock → Receptor.c*).
- Create the *Screening* subdirectory, go inside it.
- Put *1HPV.pdbqt* in the file requester and click *Save*.
- When the message *Do you want run AutoGrid ?* is shown, click *Yes* and wait the end of the calculation.
- The calculation is finish, when the AutoGrid console window disappears.

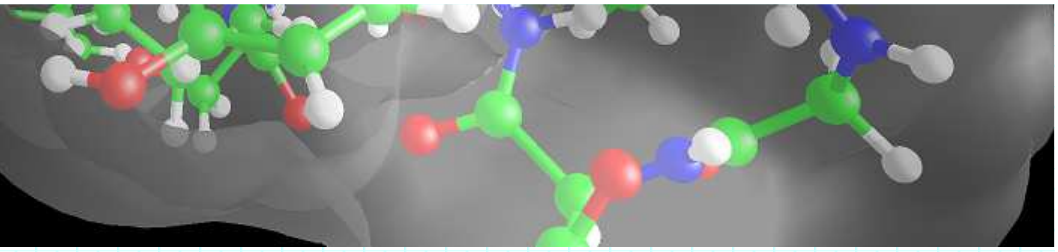
Database download



GriDock requires one or more databases that must manageable by VEGA (SDF or Zip format) and they must contain the 3D structures of the molecules that you want to screen. You can build your own databases using the tools included in VEGA ZZ, you can convert a database from 2D to 3D through the *Database 2D to 3D.c* script or you can download one from Web sites as Ligand.Info: Small-Molecule Meta-Database (*http://ligand.info*). In this tutorial, a small database will be downloaded from the that Web site.

- Connect to *http://ligand.info* with your preferred Web browser.
- Download the *ChemBank* subset in zip format. This database is small and contains 2,344 molecules.
- Unzip the archive in the *Screening* directory.
- Rename the *ligand_info_subset_1.sdf* file to *ChemBank.sdf*.

Database check



If you want check the database, you can open it with VEGA ZZ:

- In the main menu, select *File → Database → Open*, go to the *Screening* directory and double click on *ChemBank.sdf*. The *Database explorer* window will be shown.
- To extract/visualize one molecule, just double click on its name in the Molecule list or click on the name and click the *Get* button (the return key has the same function).
- To close the database, click on *ChemBank.sdf* in the *Database* column and press the *Close* button.

Evaluation of the starting complex[1]

In order to compare the screening results with the co-crystallized ligand, it may be interesting to evaluate the complex interaction energy. As first step, you need to extract the ligand from the crystal structure.

- In VEGA ZZ, open the refined structure (*1HPV_min.iff* file).
- Remove the water (*Edit → Remove → Water*).
- Remove the first two segments (*Edit → Remove → Segment*). Now in the workspace, only the ligand is present.
- Run the *AutoDock\Ligand.c* script and save the ligand as *Ligand.pdbqt* in the *Screening* directory.

WARNING:

Don't normalize the ligand atom coordinates because you need to preserve the starting position.

Evaluation of the starting complex[2]

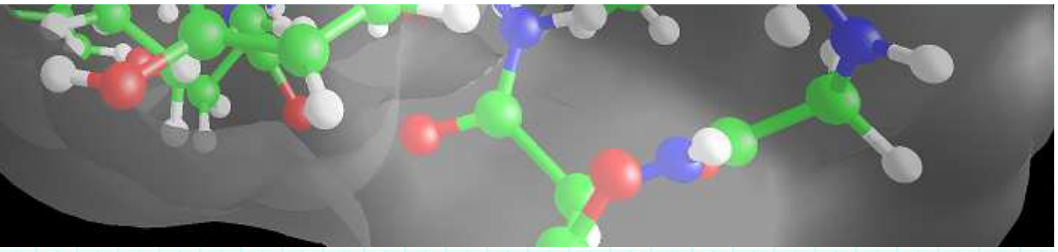
To calculate the interaction energy between the co-crystallized ligand and the HIV-1 protease:

- Open the VEGA console (*Start → VEGA ZZ → VEGA* console).
- Change the current directory to the working directory (*Screening*) by the *cd* command.
- In the console type:

```
gridock -t score.dpf 1HPV.pdbqt Ligand.pdbqt
```

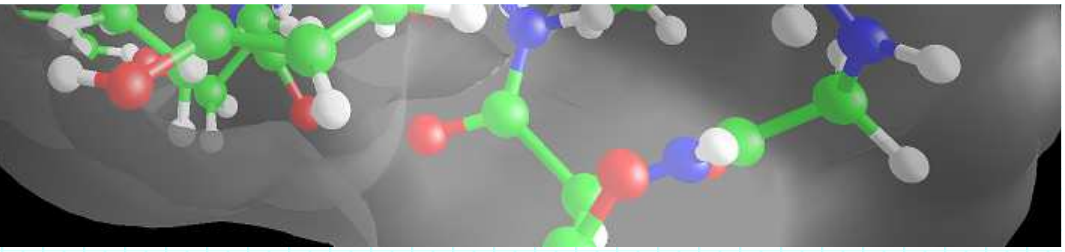
- the *-t* option is used to select another input template file for AutoDock 4. The *score.dpf* template keeps the starting position and conformation of the ligand and evaluate the interaction energy.
- When the calculation is finished, looking inside the log or the *1HPV_Ligand.csv* file, it's possible to read the interaction energy and the *Ki* of the best complex.

Log output file



```
16:31:33 ************************
16:31:33 INIT: GriDock 1.0.0.21 started on Windows
16:31:33 INIT: Local time Mon, 09 Mar 2009 17:31:33
16:31:33 INIT: Cpu model: AMD Athlon(tm) MP 2200+
16:31:33 INIT: CPUs/Cores detected: 2
16:31:33 INIT: CPUs/Cores used: 1
16:31:33 INIT: AutoDock/VEGA directory: "X:\Lcc\Vega"
16:31:33 INIT: AutoDock executable: "X:\Lcc\Vega\AutoDock4.exe"
16:31:33 INIT: VEGA executable: "X:\Lcc\Vega\Vega.exe"
16:31:33 INIT: Receptor file: "1HPV.pdbqt"
16:31:33 INIT: Database file: "Ligand.pdbqt"
16:31:33 INIT: AutoDock template file: "X:\Lcc\Vega\Data\Autodock\score.dpf"
16:31:33 INIT: AutoDock output archive: "1HPV-Ligand_01.zip"
16:31:33 INIT: Max. size of AutoDock output archive: 4000000000 bytes
16:31:33 INIT: Energy output file: "1HPV-Ligand.csv"
16:31:33 INIT: Energy output delay: 300 sec.
16:31:33 INIT: Temporary file directory: "C:\DOCUME~1\ADMINI~1\IMPOST~1\Temp"
16:31:33 INIT: First molecule to dock: 1
16:31:33 INIT: Input database in PDBQT format: one molecule only will be docked
16:31:33 INIT: AMMP time-out: 120 sec.
16:31:33 INIT: AutoDock time-out: 12000 sec.
16:31:33 INIT: VEGA time-out: 120 sec.
16:31:34 INFO: Starting AutoDock - Molecule 1 (Ligand)
16:31:36 INFO: Molecule 1 - Docking finished (0m 2s)
16:31:36 DOCK: Molecule 1 - Best model 1, Best Binding energy = -8.55 kcal/mol, Ki = 543.74 uM
16:31:36 INFO: Energy file updated
16:31:36 INFO: End of calculation
16:31:36 INFO: Docked molecules 1
16:31:36 INFO: Elapsed time 0h 0m 3s
```

CSV output file



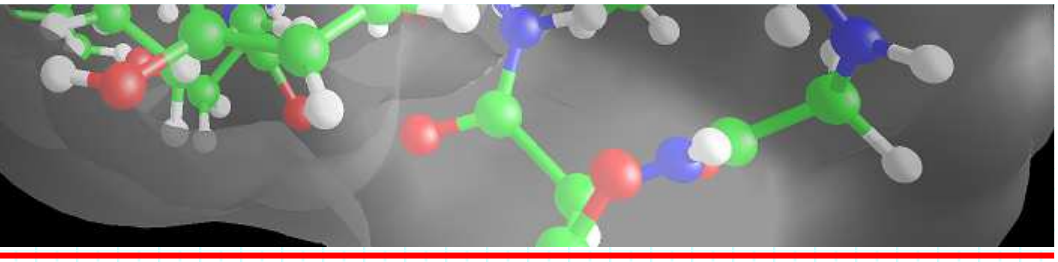
The same information van be found in the *1HPV_Ligand.csv* file, but it's formatted to be managed by a spreadsheet (e.g. Excel):

```
Database; MolID; Pose; Ki; Binding; Intermolecular; VdW + Hbond + Desolv; Electrostatic;
  Internal; Torsional; Unbound; Molecule name
Ligand; 1; 1; 543,74; -8,55; -10,26; -9,63; -0,62; -1,31; 3,02; 0,00; Ligand
```

If you want visualize the resulting complex:

- Extract the file *1HPV-Ligand_00000001.dlg* from the *1HPV-Ligand_01.zip* archive to the Screening directory.
- Open the extracted file with VEGA ZZ. The *Trajectory analysis* window is shown, but you can't do anything because only one complex is included in the output.
- Close the *Trajectory analysis* window, clicking the *Cancel* button.
- To highlight the ligand, color the structure by molecule (*View → Color → By molecule*): the ligand will be painted in red and the enzyme in white.

Running the screening



- To start the virtual screening, type in the command prompt:

```
gridock 1HPV.pdbqt ChemBank.sdf
```

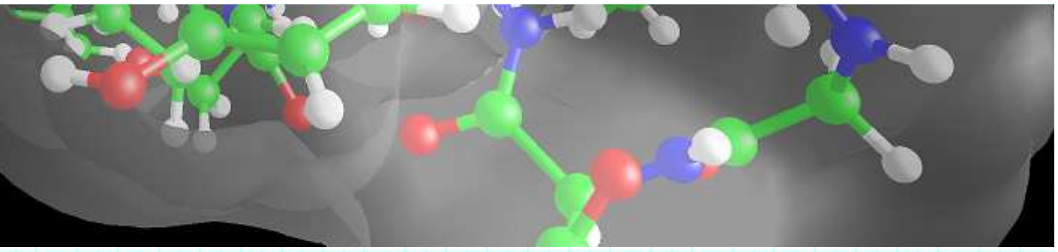
- and hit return. The time required to screen all 2,344 molecules contained in the ChemBank.sdf database depends on the computational power of your system. It's possible to check the calculation progress, viewing the log file (*gridock_YYYYMMDD.log*) with a text editor.
- If you want to screen a limited number of molecules and not the entire database, you can specify the starting and the ending molecule numbers:

```
gridock -f 10 -l 200 1HPV.pdbqt ChemBank.sdf
```

the molecules in the range from *10* to *200* will be screened only. If you want start from the first molecule, the *-f* option can be omitted.

```
gridock -l 200 1HPV.pdbqt ChemBank.sdf
```

Results



- You can consider ligands as potential HIV-1 protease inhibitors when the binding energy and/or the binding constants (Ki) are respectively less than *-8.55 kcal/mol* and *543,74 μM*.
- Considering the molecules from 1 to 200 in the ChemBank database and analyzing the *1HPV-ChemBank.csv* file, you can obtain these results:

```
Database; MolID; Pose; Ki; Binding; Intermolecular; VdW + Hbond + Desolv; Electrostatic; Internal; Torsional;
  Unbound; Molecule name
ChemBank; 123; 10; 54,31; -9,91; -9,91; -9,69; -0,22; 0,00; 0,00; 0,00; Paxilline
ChemBank; 12; 7; 606,27; -8,48; -9,10; -8,99; -0,11; -1,20; 1,10; -0,71; BML-190
ChemBank; 151; 2; 747,69; -8,36; -8,84; -8,67; -0,17; -0,39; 0,55; -0,33; Go6976
ChemBank; 131; 4; 795,11; -8,32; -8,75; -7,59; -1,16; -0,35; 0,55; -0,23; AM-580
ChemBank; 101; 4; 1,07; -8,15; -8,91; -8,90; -0,01; -0,65; 0,82; -0,59; 24,25-Dihydroxyvitamin D3
ChemBank; 75; 4; 1,08; -8,14; -8,14; -8,16; 0,02; 0,00; 0,00; 0,00; Grayanotoxin III
ChemBank; 125; 3; 1,54; -7,93; -8,44; -8,17; -0,28; -0,41; 0,55; -0,38; PCO-400
ChemBank; 133; 2; 1,79; -7,84; -8,44; -8,13; -0,31; -0,36; 0,55; -0,42; TTNPB
ChemBank; 97; 10; 2,02; -7,77; -8,83; -8,78; -0,05; -0,35; 0,82; -0,59; 25-Hydroxyvitamin D3
ChemBank; 99; 9; 2,20; -7,72; -8,46; -8,42; -0,04; -0,66; 0,82; -0,58; 1,25-Dihydroxyvitamin D3
ChemBank; 31; 9; 2,45; -7,65; -7,65; -7,57; -0,08; 0,00; 0,00; 0,00; Cyclopiazonic Acid
ChemBank; 119; 1; 2,51; -7,64; -7,64; -7,67; 0,03; 0,00; 0,00; 0,00; 6-Formylindolo [3,2B] Carbazole
ChemBank; 107; 8; 2,76; -7,58; -8,62; -8,05; -0,57; -0,70; 1,10; -0,64; 13-Cis Retinoic Acid
```