fpocket Users' Manual

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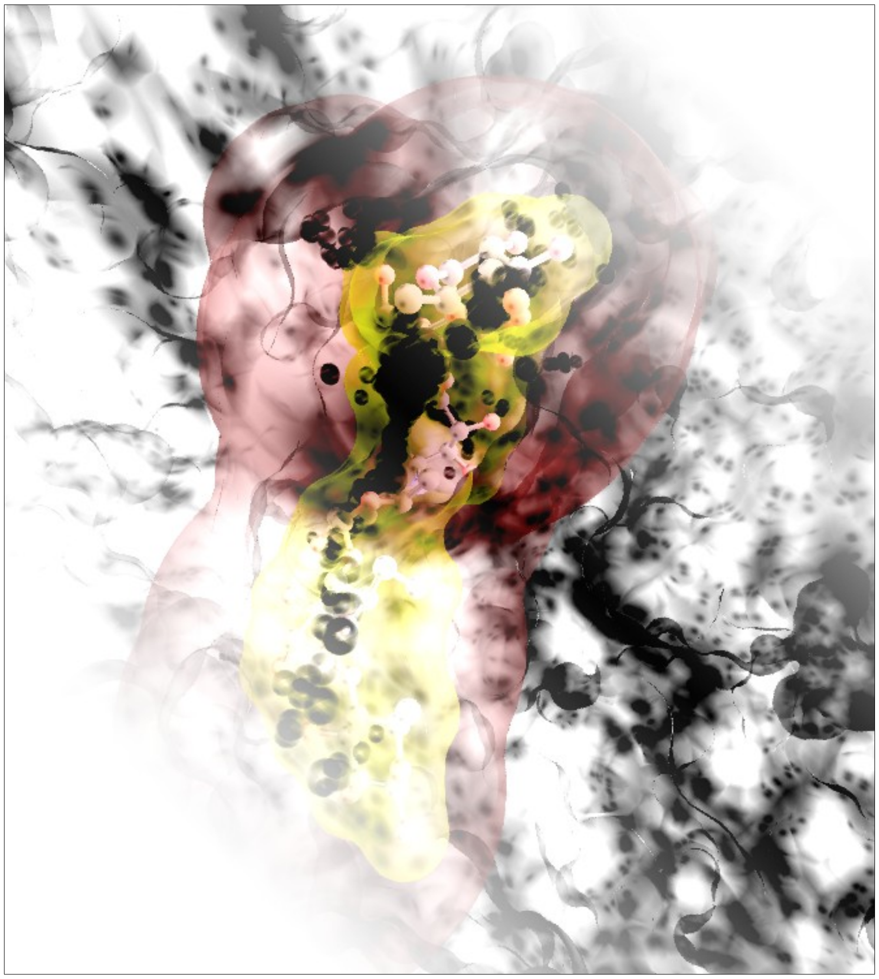
fpocket is a protein pocket prediction algorithm. Given a PDB protein structure it enables the user to identify potent binding sites. Based on Voronoi tessellation, this algorithm is very fast and particularly well suited for large scale protein binding pocket screenings and development of scoring functions for binding pocket characterization.



acarbose binding site on alpha amylase (7taa).picture generated using VMD and tachyon rendering and GIMP post-processing.

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Notes

1. *This program uses output coming from Qhull. Qhull is currently not shipped with fpocket and has to be installed seperately. More information about Qhull can be found in the paper : Barber, C.B., Dobkin, D.P., and Huhdanpaa, H.T., "The Quickhull algorithm for convex hulls," ACM Trans. on Mathematical Software, 22(4):469-483, Dec 1996, http://www.qhull.org*

2. *This software includes code developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign. The PDB parser of the Molfile Plugin of VMD were modified for the purposes of fpocket's PDB parsing.*

3. *Within the whole documentation code and output from computer programs are represented and formatted in the following way :* `ls -1 > out.txt`

Contents

Introduction

Thanks for taking the time to read this official users' guide of *fpocket*. In this guide are presented general functionalities of the *fpocket* program and its derivatives, *dpocket* and *tpocket*. Yes, indeed fpocket is a package of three distinct programs, mentioned here before. fpocket is an acronym for “free” pocket, as fpocket is distributed under the GNU GPL; dpocket is an acronym for “describing” pockets as it is for extraction of physico-chemical descriptors of pockets; tpocket is an acronym for “testing” pockets, as it is for testing on a large scale scoring functions for ranking protein cavities among each other.

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Vincent Le Guilloux, Peter Schmidtke and Pierre Tufféry disclaim all copyright interests of fpocket, dpocket and tpocket (which perform protein cavity detection, cavity descriptor extraction, large scale cavity prediction evaluations, respectively), written by Vincent Le Guilloux and Peter Schmidtke.

Installation

Prerequisites

Currently fpocket proposes two different ways for visualization of binding pockets. Both are based on commonly used molecular visualization tools : VMD[REF] and PyMol[REF]. In order to use visualization you need to install at least one of both softwares. Currently, visualization using VMD has better rendering and performances and visualization using PyMol better handling of binding pockets. You can download VMD for free from http://www.ks.uiuc.edu/Research/vmd/. PyMol can be freely downloaded from http://delsci.com/rel/099/.

Dependencies

fpocket relies on Qhull[REF]. This software computes the convex hull, Delaunay triangulation, Voronoi diagram, halfspace intersection about a point, furthest-site Delaunay triangulation, and furthest-site Voronoi diagram. For more information and for downloading Qhull refer to http://www.qhull.org. Qhull is free software. Unfortunately, qhull is currently not distributed with fpocket. So you have to install it separately on your desktop and enable the *qvoronoi* program to be executable in a normal shell (bash, csh...).

Please test, if qhull works properly using the following procedure :

1. open a new shell
2. type the following : **`rbox c P0 D2 | qvoronoi Fo`**

If you obtain the following result, your installation of qhull was correct :

```
4
5 1 2     -1      0   -0.5
5 1 3      1     -0     -0
5 2 4      1     -0     -0
5 3 4     -1      0    0.5
```

If you obtain a segmentation fault, please refer to known bugs section of this manual.

Qhull is only required upon execution of fpocket, tpocket and dpocket, but not necessary for compilation of fpocket, dpocket and tpocket. The version Qhull 2003.1 was tested during the development of this release and should perform well for fpocket's purposes. The GNU C compiler version 3.4.6 was used to compile Qhull. See known bugs for possible compilation/execution issues with Qhull.

System Requirements

fpocket is available for Linux/Unix type systems only. Although it hasn't been tested on Mac OS X for now, it should also compile and work nicely on this system type. fpocket is currently not available for Windows systems.

In order to run fpocket, you should have at minimum a Pentium III 500 Mhz with 512Mb of RAM. This program was co-developed and tested under the following Linux distributions : openSuse 10.3, Centos 5.2, Fedora Core 7, Ubuntu 7.1.

How to install fpocket

j'attends le ./configure etc...pour voir commenton pourra faire...

Known Bugs

A known bug exists for Qhull using some newer versions of gcc. Qhull under some conditions returns a segmentation fault during execution, which would render fpocket unusable. If you issue this kind of bug, please do the following modification in the Makefile of Qhull.

Replace the occurrence of **`CFLAGS = -g -O2`** in this file to **`CFLAGS = -g -O`**.

Recompile Qhull using the **`./configure ; make; make install`** commands and normally Qhull should work properly. For more Qhull secific questions and bugs, please refer to Qhull directly at qhull@qhull.org or on the website http://www.qhull.org.

Getting Started

fpocket

Example

Here is shown a very simple and straightforward example of how to run fpocket on a single PDB file downloaded from the RCSB PDB[REF]. The following line will execute fpocket on the **`3LKF.pdb`** file in the **`path_to_file`** directory.

```
fpocket -f path_to_file/3LKF.pdb
```

It is mandatory to give a PDB input file using the **`-f`** flag in command line. If nothing is given, fpocket prints the fpocket usage/help to the screen. fpocket will use standard parameters for the detection of cavities. Fore more information about these parameters see the Advanded features chapter – fpocket section (page 8).

If fpocket works properly the output on the screen should look like this :

```
=========== Pocket hunting begins ==========
> Freeing remainnig memory...
=========== Pocket hunting ends ==========
```

If you have a look now in the **`path_to_file`** directory, you will notice that fpocket created a folder named **`3LKF_out/`**. This folder contains all the output from fpocket, so what you are actually interested in. If you just want to see rapidly the results, go to the **`3LKF_out`** directory and launch the **`3LKF_VMD.sh`** script. This script will launch the VMD molecular visualizer and charge the protein with binding site information coming from fpocket. CONTINUE HERE

Input

Mandatory:

1: flag -f : one standard PDB file,

Optional:

For this see Advanced features chapter – fpocket section (page 8).

Output

fpocket yields output directly in the directory of the data file. The **`ll 3LKF_out`** output of the current sample run would look something like this:

```
total 332
-rw-r--r-- 1 peter users    769 Nov 29 00:14 3LKF.pml
-rw-r--r-- 1 peter users    698 Nov 29 00:14 3LKF.tcl
-rwxr-xr-x 1 peter users     30 Nov 29 00:14 3LKF_PYMOL.sh
-rwxr-xr-x 1 peter users     41 Nov 29 00:14 3LKF_VMD.sh
-rw-r--r-- 1 peter users 245835 Nov 29 00:14 3LKF_out.pdb
-rw-r--r-- 1 peter users   6725 Nov 29 00:14 3LKF_pockets.info
-rw-r--r-- 1 peter users  49355 Nov 29 00:14 3LKF_pockets.pqr
drwxr-xr-x 2 peter users   4096 Nov 29 00:14 pockets
```

As you can see, fpocket provides a lot of files and another subdirectory. However, majority of these files are necessary for easy visualization of binding pockets. Lets explain the content and utility of each file :

- **`3LKF.pml`** : this is a PyMOL script for visualization of binding pockets using PyMOL
- **`3LKF.tcl`** : this a tcl script for visualization of binding pockets using VMD
- **`3LKF_PYMOL.sh`** : this is the executable script to launch fast visualization using PYMOL
- **`3LKF_VMD.sh`** : this is the executable script to launch fast visualization using VMD
- **`3LKF_out.pdb`** : this is the most important file, it contains the initial PDB structure given as input. Non cofactor HETATM occurrences will be stripped of in this file compared to the original PDB input file. The PDB file contains centers of alpha spheres using the HETATM definition as dummy atoms. These alpha sphere centers are attached in the end of the PDB file, using the STP residue name (for site point). Apolar alpha spheres carry the atom name APOL, polar alpha spheres the atom name POL. Pockets are sets of alpha spheres. They can be distinguished by residue number. Thus residue STP 1 would be the first binding pocket according to fpocket. To show this more clearly here is an extract of the **`3LKF_out.pdb`** :

```
ATOM   2349   CD LYS A 299       9.679  16.827 105.636  1.00 19.91           C
ATOM   2350   CE LYS A 299      10.371  16.314 104.370  1.00 25.17           C
ATOM   2351   NZ LYS A 299      11.749  15.794 104.597  1.00 32.36           N
ATOM   2352  OXT LYS A 299       5.240  20.009 107.670  1.00 16.06           O
HETATM 2736  POL STP C   1      18.291  37.420  83.622  0.00  0.00          Ve
HETATM 2756  POL STP C   1      18.445  37.638  83.606  0.00  0.00          Ve
HETATM 3208  POL STP C   1      18.325  37.403  83.631  0.00  0.00          Ve
HETATM 3208  POL STP C   1      18.450  37.618  83.610  0.00  0.00          Ve
```

- **`3LKF_pockets.info`** : this file contains more human readable information about results after pocket detection. It contains the score associated at each pocket found, as well as all the descriptors of the pocket. The sample gives the following output for the first ranked binding pocket :

```
      ## FPOCKET RESULTS ##

## POCKET 1 ##
        0  - Pocket Score:               86.1249
        1  - Number of Voronoi vertices:  163
        2  - Mean alpha-sphere radius:    3.786007
```

```
3  - Mean alpha-sphere solvent accessibility: 0.495167
4  - Flexibility:                    0.277080
5  - Hydrophobicity Score:           24.393940
6  - Polarity Score:                 16
7  - Volume Score:                   4.030303
8  - Real volume (approximation): 4128.224121
9  - Charge Score:                   2
10 - Local hydrophobic density Score: 19.434782
11 - Number of apolar alpha sphere: 46
12 - Amino acid composition:
12 -   0   0   0   3   3   1   2   3   2   3   2   5   2   2
       0   2   1   0   0   2
```

- **`3LKF_pockets.pqr`** : This file contains all alpha sphere centers, as the 3LKF_out.pdb file, but contains no information about the protein structure. Furthermore using the pqr format enables writing of the van der Waals radius of atoms explicitely in this file. Here this possibility was used to output the radii of alpha spheres of a pocket. Charging this pqr file, one can analyze more precisely the volume recognized by fpocket. Note that, currently only VMD supports reading this format correctly. PyMOL is able to read pqr file, but does not interpret van der Waals radii.
- **`pockets/`** : Well, again a subdirectory. But I promise, it's the last one. For development purposes or easy analysis, fpocket proposes this directory which contains according to the current example :

```
pocket0_atm.pdb   pocket2_vert.pqr  pocket5_atm.pdb   pocket7_vert.pqr
pocket0_vert.pqr  pocket3_atm.pdb   pocket5_vert.pqr  pocket8_atm.pdb
pocket1_atm.pdb   pocket3_vert.pqr  pocket6_atm.pdb   pocket8_vert.pqr
pocket1_vert.pqr  pocket4_atm.pdb   pocket6_vert.pqr  pocket9_atm.pdb
pocket2_atm.pdb   pocket4_vert.pqr  pocket7_atm.pdb   pocket9_vert.pqr
```

The **`*_atm.pdb`** files contain only the atoms contacted by alpha spheres in the given pocket. Complementary to this information, **`*_vert.pqr`** files contain only the centers and radii of alpha spheres within the respective pocket. As extensions mention, atoms are output in the PDB file format and alpha sphere centers in the PQR file format.

Is there something else? No, you have done. Congratulations, you have successfully performed your first cavity prediction with fpocket...without any accidents we hope. As you might have seen, usage of fpocket is rather simple, although it is command line based software (for now). Furthermore you should have seen that fpocket is very fast, well, lets say if you do not run a P1 100Mhz.

As mentioned before, fpocket provides much more possibilities especially for filtering out unwanted pockets, clustering of alpha spheres. For all these issues and usage of these more advanced features, refer to chapter Advanced features, section fpocket (page 8) of this manual.

dpocket

Until now you have seen what the majority of cavity detection algorithms can do. So a part from speed and hopefully prediction results, nothing distinguishes fpocket from other algorithms like ligsite, sitemap, sitefinder, pocketpicker, pass .... etc...

This is just partially true, because the fpocket package contains dpocket. D is an acronym for describing. One purpose a cavity detection algorithm can be used for is for extraction of descriptors of the physico-chemical environment of the cavity. dpocket allows to do this in a very simple and straightforward way. As extracting binding pocket descriptors on only one protein would be somehow meaningless for studying pocket characteristics, dpocket enables analysis of multiple structures. So now, no longer scripting and automation is necessary to do these kind of things. But lets have a closer look using again a very simple example you can try on your workstation.

Example

Input

Output

tpocket

Advanced features

fpocket

Tpocket

Dpocket

Writing your own scoring function

Writing your own descriptor

Samples

Sample Tpocket input

Sample Dpocket input