

## Overview

FLIRT (FLexible InteRaction Tool) is an analysis script for implementation with VMD software to rapidly (seconds to minutes) analyse general contacts, hydrogen bonds, cation- $\pi$  interactions and hydrophobic (non-polar) contacts for a single PDB file, or over entire MD trajectories (thousands of snapshots). Interactions between atoms of two selections, within user-specified geometric constraints (i.e., cutoff distances and angles), are tallied on a per-atom basis and accumulated into any desired attribute (i.e., VMD attributes are index, resid, serial, residue, resname, type, name, structure) for the first selection, or decomposed into pairwise interactions with any attribute of a second selection. The results for either accumulated or pairwise measurements may be summarized as a mean and standard deviation over all frames analysed (\*.xy.dat and \*.pairxy.dat files, respectively) or time-resolved over an entire trajectory (\*.xyz.dat and \*.pairxyz.dat files, respectively).

## Usage

The script (flirt.tcl) must be copied into the working directory and loaded into VMD using the Tk Console and command 'source flirt.tcl'. A measurement may be executed by typing 'flirt *measurement* [*options*]' into the console, selecting only ONE *measurement* and any number of *options*, described below:

Measurement modes (*measurement*):

- **-contact *selection1 selection2 cutoff***

Extension of the 'measure contacts' command shipped with VMD. Atom pairs between *selection1* and *selection2*, within *cutoff* distance (Å), are detected and summarised according to the *options* provided

- **-hbond *selection1 selection2 cutoff angle***

Extension of the 'measure hbonds' command shipped with VMD. A hydrogen bond is detected if the distance between donor and acceptor (non-hydrogen) atoms is within the *cutoff* distance (Å), and the donor-hydrogen-acceptor angle

is less than *angle* from 180 degrees. Values of 3 Å and 20° are typical. By default, a hydrogen bond is only detected if the donor and acceptor atoms are part of *selection1* and *selection2*, respectively. The ‘-combine’ option may be specified if both donor and acceptor interactions are to be tallied regardless of selection order. It is also a known issue in VMD that ‘*hbond*’ measurements may produce artefacts if *selection1* and *selection2* share a common set of atoms. In this case, the ‘-exclude *attribute*’ option may be used. For example, ‘-exclude resid’ will ignore all hydrogen bonds where donors and acceptor atoms share the same residue ID number.

- **-cationpi *selection1 selection2 cutoff1 cutoff2***

A cation- $\pi$  interaction is detected between *selection1* and *selection2* if all carbons of a six-membered ring system are within *cutoff1* (Å) to a cationic centre, and if distances measured for all carbons differ no greater than *cutoff2* (Å). Values of 7 Å and 1.5 Å for *cutoff1* and *cutoff2*, respectively, have been used by (Grauffel et al., 2013) to approximate the maximum interaction distance and a 120° selection cone for the cation position with respect to the plane of the ring system (Minoux and Chipot, 1999, Petersen et al., 2005). Ring systems that are not part of TRP, TYR or PHE residues, and cations that are not part of ARG, LYS, DPC or ASM residues, must be defined in the ‘*proc flirt::check\_cationpi*’ section of the code for the interaction to be detected (note that the code in its current state doesn’t allow for residues which have multiple ring systems). By default, *selection1* is scanned for  $\pi$ -systems and *selection2* for cations, but, again, this condition may be relaxed using the ‘-combine’ option. Also, a ‘*measure\_cationpi cutoff1 cutoff2 selection1 selection2*’ procedure is made available, which returns a list of  $\pi$ -atom indices (six unique entries) from *selection1* and paired cations for *selection2* (atom index is entered six times to match size of first list).

- **-nonpolar *selection1 selection2 cutoff***

Is a modification of the ‘-contacts’ measurement, but ignores any interaction from atoms that are not assigned as a nonpolar carbon or hydrogen. This function requires that the ‘*flirt::check\_hydrophobic*’ procedure is updated with any non-standard residues (i.e., not a common amino acid). Also, a

'measure\_nonpolar cutoff sel1 sel2' procedure is introduced, which outputs non-polar contacts in a selected frame. Atom indices of *selection1* are in the first list alongside paired atoms of *selection2* in a second list.

Options ([*options*]) may be specified in any order and include:

- **-attr1 attribute1** (default resid)  
Interactions involving atoms of *selection1* are accumulated into a shared *attribute1* every frame. Supported attributes include: index, resid, serial, residue, resname, type, name and structure.
- **-attr2 attribute2** (default resid)  
Accumulate interactions involving *selection2* atoms by *attribute2*. Nothing is done unless -pairxy and/or -pairxyz are also stated.
- **-prefix prefix** (defaults 'total', 'contacts', 'hbonds', 'cationpi' or 'nonpolar')  
Files outputted by the '-xy', '-xyz', '-pairxy', '-pairxyz' and '-total' flags are named with a prefix *prefix*.
- **-xy**  
Outputs a file '*prefix.xy.dat*' with the *attribute1(s)* of *selection1* (first column), the mean number of interactions per frame (second column) and standard deviations (third column).
- **-xyz**  
Outputs a file '*prefix.xyz.dat*' with frame numbers (first column), *attribute1(s)* of *selection1* (second column) and the number of interactions per frame (third column). A blank line is inserted to create a new block of data for every frame for compatibility with the pm3d 3D-plotting function of Gnuplot.
- **-pairxy**  
Outputs a file '*prefix.pairxy.dat*' of summarised pairwise interactions between *attribute1(s)* of *selection1* (first column) and *attribute2(s)* of *selection2* (second column) according to the mean number of interactions per frame (third column) and standard deviation (fourth column).
- **-pairxyz**  
Outputs a file '*prefix.pairxyz.dat*' with frame numbers (first column), pairwise interactions between *selection1 attribute1(s)* and *selection2 attribute2(s)*

(second column, formatted as *attribute1.attribute2*) and the number of interactions per frame (third column).

- **-total**

Outputs a file '*prefix.total.dat*' of the total interactions detected between atoms *selection1* and *selection2* for every frame.

- **-start *frame*** (default 0)

- **-stop *frame*** (default last frame of trajectory)

- **-skip *frame*** (default 1)

- **-betas**

A beta value, useful for colour scaling, is assigned to each atom in *selection1* according to the average number of interactions to its corresponding *attribute1* (requires option '-attr1 *attribute1*').

- **-zeros**

By default, no value is written to '*.xy.dat*' and '*.xyz.dat*' files if no interactions are detected. If zero values are necessary, as is the case for some plotting programs, they can be written by specifying this flag. This flag is ignored for pairwise outputs.

- **-combine**

Is used to consider both donor and acceptor contributions to hydrogen bonding by *selection1* and *selection2*. May also be used to detect cation- $\pi$  interactions where both selections contain  $\pi$ -systems and cations. This flag is ignored for '-contact' and '-nonpolar' *measurements*.

- **-exclude *attribute***

Interactions are ignored when atoms of *selection1* and *selection2* share the same *attribute*.

- **-mol *mol*** (default top)

Specify this flag only if the molecule isn't 'top'

## References

- GRAUFFEL, C., YANG, B., HE, T., ROBERTS, M. F., GERSHENSON, A. & REUTER, N. 2013. Cation- $\pi$  interactions as lipid-specific anchors for phosphatidylinositol-specific phospholipase C. *Journal of the American Chemical Society*, 135, 5740-5750.
- MINOUX, H. & CHIPOT, C. 1999. Cation- $\pi$  interactions in proteins: Can simple models provide an accurate description? *Journal of the American Chemical Society*, 121, 10366-10372.
- PETERSEN, F. N. R., JENSEN, M. Ø. & NIELSEN, C. H. 2005. Interfacial tryptophan residues: A role for the cation- $\pi$  effect? *Biophysical Journal*, 89, 3985-3996.