FlexS Command Line Documentation
Version 5.6

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1 Introduction

All links, references, table of contents lines etc. in this document are clickable.

FlexS is a powerful command line tool for the 3D alignment and superposition of molecules.[3] For a given pair of ligands, FlexS predicts the conformation and orientation of one of the ligands relative to the other one. 3D ligand alignment is often the method of choice when the structure of the receptor or target is unknown, but sufficient information about a binding ligand is available, e.g. from biochemical assays.
FlexS is also a "component" within our flagship 3D modeling platform SeeSAR that has been conceived for drug researchers of any discipline and educational level. Within SeeSAR's Similarity Scanner Mode, you can easily execute 3D ligand alignments with FlexS and directly visualize and inspect the results. Furthermore, you can conveniently add pharmacophore constraints to guide the alignment step and receive more relevant results.

FlexS at the command line can be used for:

- Alignment and superposition of molecules in 3D
- Preparation of compounds for Quantitative Structure Activity Relationship (QSAR) analysis studies
- Straightforward identification of scaffold/compound mimics
- Performing ligand-based virtual screening
- Consideration of pharmacophore constraints *during* the alignment step (i.e., the pharmacophores are not acting as post-filters)
- Optional enumeration of stereo isomers during the superposition
- Processing of unlimited ligand numbers

Please note that this package is a command line package.

Background FlexS uses an incremental construction procedure for superposing two ligands.[3] The ligand to be superimposed is partitioned into fragments and is treated as flexible. The other ligand, the so called reference ligand, is kept rigid. An initial fragment (anchor fragment, rigid-body placement procedure)[1] of the flexible ligand is placed and superimposed onto a part of the reference ligand and, subsequently, the remaining fragments are added in a step-wise manner while taking the conformational degrees of freedom into account. The sequence in which fragments are added is thereby selected dynamically depending on the actual placement. Molecular flexibility is handled using discrete sets of torsional angles for each rotatable bond and sets of distinct ring conformations. In addition, putative intermolecular interactions with a possible/hypothetical receptor are modeled by following the concept of paired intermolecular interactions.[2] The interaction geometries around functional groups are approximated by sets of discrete interaction points. This treatment allows to apply discrete combinatorial procedures for the placement of molecular fragments of the flexible ligand onto the reference ligand. Physicochemical properties are distributed across the molecules by approximating the respective densities by sets of Gaussian functions. The superimposed ligands are then ranked with a scoring function considering the matching paired intermolecular interactions with a putative receptor, the van-der-Waals overlap volume, the property overlap expressed by Gaussians and the topological matching expressed by subtrees. The overall similarity score is annotated in the output SD file (value between 0 and 1). The FlexS algorithm proved to be successful in reproducing bioactive binding conformations with a root-mean-square-deviation (rmsd) below 1.5 Å in more than 60% of the studied cases.[3]

2 Technical Prerequisites

2.1 Required Software

FlexS is a command line application. Control through a graphical user interface (GUI) is available in SeeSAR. We recommend you also install SeeSAR to swiftly carry out all sorts of preparation steps, notably any relevant pharmacophore definitions. The preparation can then be written to a `*.flexs` file and used at the command line with FlexS (this package).

It needs the following to run:

- The **FlexS package**
  (`https://biosolveit.de/download/?product=flexs`)
  Depending on your operating system, some libraries may have to be installed (get in touch with us: `mailto:support@biosolveit.com`; and please mention any errors/warnings that you see in your mail)
- A **shell** (Linux/Unix) or a terminal (macOS), or a command line environment (Windows; e.g.: cmd.exe)
- A valid **license** (from `mailto:license@biosolveit.com`)

2.2 Licensing

FlexS needs a license to operate that is available from us. There are various sorts of licenses, but in most of the cases your early testing will employ a license file that simply needs to be put next to the executable: Just drop the `*.lic` file that you received from us in the folder in which `flexs[.exe]` resides.

The license setup instructions will come with the license that we will send out — or that has already been sent out to you. In case you don't have a license yet, please get in touch with us at `mailto:contact@biosolveit.de`, and provide us with the necessary information.
Please note: A valid SeeSAR license will also be accepted by FlexS.

License File Locations A "test license" that you can request online and that is sent to you instantaneously can simply be placed next to the executable (`flexs.exe`, `FlexS` or `flexs` — depending on your operating system). For macOS please read on...

---

macOS Specialties On macOS, the executable will typically reside inside the *.app package:

```
/Applications/FlexS.app/Contents/MacOS/FlexS
```

To place the short term test license there, you will have to go into the *.app package using a right mouse click (or CTRL-click) on FlexS.app in the Finder, and click on "Show package contents". In there, you will see the Contents/ subfolder, in there the MacOS subfolder, and in there, the FlexS executable. If you are about to use the **test license**, place is right there, next to the executable. A longer term license will be handled separately, we will tell you how when we send that very license.

When you call FlexS for the first time, go to the Finder, and navigate to the Applications folder. Do a right(!) click on FlexS.app, and — if applicable — confirm that you want to open the program. It will flash up once, and you are good to go at the terminal prompt from there on.

Obtaining a License File Using `--license-info` you can obtain information about the specification of your license server machine, the searched directories, and the validity of the currently used license files. This may also be useful when FlexS is not starting up as you would expect it to.

Call FlexS with the `--license-info` option, to see an output like this:

```
.\flexs.exe --license-info
================================================================================
 License Information:
================================================================================
Host-ID: "0c379650cfdf 00ff8c8dfdc6 0093376fa5e6 0293376fa5e5 0093376fa5e5 0093376fa5e9"
BIOSOLVE_LICENSE_FILE:
LM_LICENSE_FILE:

Currently used key/path after environment variables:
C:/Users/sajung/FlexS-5.5.0

FlexS:
 >> Valid key, expires on Tuesday, 31 December 2028



Request a license:
***
*** https://www.biosolveit.de/license/?product=flexs&operating_system=win64...
***
```

Request an evaluation or longer-term license using the link that is provided at the very bottom of the output.

Also, this output may help us to find out if there are any problems with your license or its setup.

3 Jump Start: 3D Molecular Superposition

To run a 3D superposition at the command line with all defaults, you will need at least:

- A ligand input file (`.sdf`, `.mol`, `.mol2`, with 3D coordinates for every compound) containing the compounds-to-be-superposed. These compounds will be treated as flexible during the alignment.

(a) A template ligand file

- A template ligand file (`.sdf`, `.mol`, `.mol2` with 3D coordinates). This should be a single compound, ideally in a known bioactive conformation or in a low energy conformation. The template ligand will be treated as rigid during the alignment. The compounds contained in the input ligand file will be superposed on this conformation of the template ligand.

(b) A superposing definition file

- A superposing definition file (`.flexs`) that you have exported from within SeeSAR's Similarity Scanner Mode.

Now run this — with the file names replaced by your own names of course:

In case (a), that is, if you provided a template ligand file:

```
./flexs -i My3DLibrary.sdf -t My3DTemplateLigand.sdf -o MySuperposedOutput.sdf
```

In case (b), that is, if you have prepared and exported a superposing definition file from SeeSAR:

```
./flexs -i MyLibrary.sdf --superposing-definition MyDefinitionFile.flexs
-o MySuperposedOutput.sdf
```

Depending on your operating system, please certainly also adapt the command line usage, e.g., use a slash or a backslash etc.

The calls above will create an output file `MySuperposedOutput.sdf` that, by default, contains a maximum of 10 conformations for every ligand — given that the superposition was successful and the similarity to the template ligand exceeds the default threshold of 0.6 (see page 8). The SD output file will contain the similarity score in one of the SD fields (named `BIOSOLVEIT.SIMILARITY_SCORE`) for post-processing purposes.

4 Command Line Options

4.1 Overview

An overview of all command line options is available by calling FlexS with **`--help`**. Default values are bracketed:

```
./flexs -h

 Program options:
-i [ --input ] arg                    Library input molecule file. Supported file types are *.mol2, *.mol
                                      and *.sdf.
                                      Note: 3D coordinates must be provided, otherwise molecule is
                                      skipped.
-o [ --output ] arg                   Output file, only *.sdf is supported.
--superposing-definition arg          Generate superpositions on the basis of this superposing definition
                                      file (a .flexs file that can be exported from SeeSAR's Similarity
                                      Scanner mode) - it contains the template molecule and pharmacophore
                                      constraints.
                                      Note: Can't be used together with '--template'.
-t [ --template ] arg                 Template molecule file(s). Supported file types are *.mol2, *.mol
                                      and *.sdf.
                                      Multiple templates up to a maximum of 10 are supported.
                                      Note: The template molecule(s) must have 3D coordinates.
                                      Note: Can't be used together with '--superposing-definition'.

Configuration:
--allowed-6ring-confs arg (=0)        Conformations allowed for aliphatic six-membered rings:
                                           0 [Only chair conformations are allowed.]
                                           1 [Chair and twist-boat conformations are allowed.]
                                           2 [Chair, twist-boat and boat conformations are allowed.]

--keep-input-sd-tags [=arg(=1)]       Keep input sd tags for the superposition solutions.
--max-nof-conf arg (=10)              Maximum number of top-ranking result conformations for each input
                                      molecule. Note: If a superposing definition file is used, the value
                                      specified in the file is used.
--min-similarity-threshold arg (=0.6) Similarity threshold below which superpositions are discarded [0.0
                                      to 1.0]. Note: If a superposing definition file is used, the value
                                      specified in the file is used.
--stereo-mode arg (=0)                Automatically flip stereo centers during superposition:
                                           0 [Do not flip stereo centers]
                                           1 [Flip R/S stereo centers]
                                           2 [Flip E/Z stereo centers]
                                           3 [Flip R/S and E/Z stereo centers]

General options:
-h [ --help ]                         Print this help message.
--license-info                        Print license info.
--thread-count arg                    Maximum number of threads used for calculations. The default is to
                                      use all available cores.
--version                             Print version info
-v [ --verbosity ] arg (=2)           Set verbosity level
                                           0 [quiet]
                                           1 [error]
                                           2 [warning]
                                           3 [info]
                                           4 [steps]
```

The abbreviated, one-letter options are preceded with one dash **-** whereas the longer, named options are preceded with two dashes: **--**. If an option needs an argument (arg), you can include or omit the equals sign. Adapt the command line usage to your operating system and shell.

4.2 Program Options

–i [––input] arg Specify the input file in `.sdf`, `.mol` or `.mol2` format. The input file should contain all molecules which you want to superpose onto the template ligand. The input ligands will be treated as flexible during the alignment. Please make sure that all molecules have 3D coordinates, otherwise they are skipped. You can load your 2D molecule file into SeeSAR's Molecule Editor Mode and export the automatically generated 3D molecules as SD file. Alternatively, you can use the Conformator tool to generate 3D coordinates (`https://biosolveit.de/download/?product=conformator`).

Example:

```
flexs -i My3DMolecules.sdf
```

–o [––output-files] arg Specify the name of the output file (`.sdf` format). The output file will contain all successfully superposed molecules from the input file exceeding the minimal similarity threshold (defined with the `--min-similarity- threshold` option) with a maximum of conformations defined with the `--max- nof-conf` option (see Section 4.3).
One SD property field named `BIOSOLVEIT.SIMILARITY_SCORE` contains the overall similarity score (value between 0 and 1).

Example:

```
flexs -o SuperposedMolecules.sdf
```

––superposing-definition arg Specify a superposing definition file `.flexs` file. The definition file can be exported from within SeeSAR's Similarity Scanner Mode. It contains the template ligand(s) and optionally additional pharmacophore constraints that can be easily defined using SeeSAR's graphical user interface. Pharmacophores will be used to guide the alignment process and to produce only relevant conformations according to your constraints. Furthermore, selected configuration parameters are also read from the superposing definition file (see Section 4.3). Please note that the argument cannot be used together with the `--template` option.

Example:

```
flexs --superposing-definition MyDefinition.flexs
```

–t [––template] arg Specify one or more template ligand file(s). Each file can contain one or more template molecules. Supported file types are `.sdf`, `.mol` and `.mol2`. The template ligand(s) must be provided with 3D coordinates. Please note that the template ligand(s) will be considered as rigid during the superposition and alignment process. In a typical scenario, the template molecule should be given in a bioactive or low-energy conformation. The input molecules must share a sizable common substructure (five heavy atoms or more) with the template(s), otherwise they are skipped. An overall maximum of ten template molecules (either contained in a single file or in multiple separate files) can be specified at the same time.
NOTE: If you specify multiple template molecules, the maximum number of poses (specified with the option `--max-nof-conf`, see Section 4.3) is returned for each template molecule, i.e., if you have a single input molecule and two template molecules with the default number of results (=10 results), you will get 20 results in total, ten for each template molecule.
NOTE: You cannot use this option together with the `--superposing-definition` option.

Example:

```
flexs -t MyTemplate.sdf

flexs -t MyTemplate1.sdf -t MyTemplate2.sdf

flexs -t MyLowEnergyTemplates.sdf
```

4.3 Configuration

In this section, the default values for the respective option are bracketed.

--allowed-6ring-confs arg(=0) Select the conformations allowed for aliphatic, six-membered rings. By default, only low-energy chair conformations are tolerated. You can gradually adjust this behavior by allowing also the energetically less favorable twist-boat and boat conformations:

- 0 Only chair conformations are allowed. The default.
- 1 Chair and twist-boat conformations are allowed.
- 2 Chair, twist-boat and boat conformations are allowed.

Please note that if you provide a superposing definition file the parameter will be read from the respective `.flexs` file and used as default. However, you can overwrite it with the `--allowed-6ring-confs` option.

Example:

```
flexs --allowed-6ring-confs 1
```

--keep-input-sd-tags For every molecule in the input SD file the associated SD tags are read and added to every aligned pose generated for that molecule in the output SD file.
NOTE: Requires an input SD file.

Example:

```
flexs --keep-input-sd-tags
```

--max-nof-conf arg(=10) With this argument, you can control the maximum number of conformations per input ligand that will be generated and written to the output file. The default value is 10 conformations per ligand. Please note that if you provide a `.flexs` file using the `--superposing-definition` option the number of conformations will be read from the file and used as default. However, you can overwrite it with the `--max-nof-conf` option.

Example:

```
flexs --max-nof-conf 30
```

--min-similarity-threshold arg(=0.6) Specify the minimal similarity threshold. This limits the results written to the output file to those exceeding the minimal similarity (according to the FlexS similarity

score, annotated in the output SD File). The value given must be between 0 (dissimilar) and 1 (similar). All superpositions below this threshold will be discarded. The default value is 0.6. Please note that if you provide a superposing definition file using the `--superposing-definition` option the minimal similarity threshold will be read from the respective `.flexs` file and used as default. However, you can overwrite it with the `--min-similarity-threshold` option.

Example:

```
flexs --min-similarity-threshold 0.8
```

––stereo-mode arg(=0) This option allows you to control the treatment of stereo centers during the alignment by giving an integer as argument. The default value is 0, i.e., stereocenters are maintained during the superposition. The following behavior can be selected:

- 0 Do not flip stereo centers. All stereocenters of the input molecules are maintained. This is the default value.
- 1 Flip R/S stereo centers.
- 2 Flip E/Z stereo centers.
- 3 Flip both R/S and E/Z stereo centers.

Flipping R/S and/or E/Z stereo centers may help to identify the isomer with the most promising geometry for superposing onto the template ligand. Keep in mind that flipping every stereo center can lead to a combinatorial explosion for compounds with multiple stereo centers! This can lead to extended runtimes for large ligand libraries! Please note that if you provide a superposing definition file the parameter will be read from the respective `.flexs` file and used as default. However, you can overwrite it with the `---stereo-mode` option.
NOTE: If you activate E/Z flips (stereo mode 2 and 3), the algorithm will try to flip cis amides to a trans conformation if it can (but not vice versa)!

Example:

```
flexs --stereo-mode 1
```

4.4 General Options

–h [––help] Displays the command line help with short descriptions for every argument option. For more information see Section 4.1.

Example:

```
flexs --help
```

––license-info Shows command line information about the license setup you currently use. If you have any problems with your license, send an email to `mailto:support@biosolveit.com` and include this information. For more information see Section 2.2.

Example:

```
flexs --license-info
```

––thread-count arg Specify the maximum number of threads used for the superposition run. By default, all available logical cores of your computer are used. You may want to reduce the number of threads if you want to run other computations on your computer at the same time, or if you share the compute resource.

Example:

```
flexs --thread-count 4
```

––version Displays information on the version of FlexS on the command line. In quoting FlexS, please mention this version number.

Example:

```
flexs --version
```

–v [––verbosity] arg(=2) You can set the verbosity level, e.g. the level of console output, by giving an integer as argument. The default value is 2. The following options are available:

- `0` Quiet. No messages will be displayed on the console. Errors will be ignored whenever possible.
- `1` Error. Only error messages will be displayed.
- `2` Warning. The default setting, warnings and error messages will be displayed.
- `3` Info. Additional information beyond errors and warnings are displayed.
- `4` Steps. In addition to the 'Info' option, the progress is displayed in detail.

Example:

```
flexs -v 0
```

5 Further Reading, References

The original ideas behind the FlexS incremental construction algorithm are covered in the original publication by Christian Lemmen.[3] Over the years, FlexS has undergone steady further developments and improvements, for example, parallelization and pharmacophore-guided superpositions. For more information, see the additional literature below. More information on the tool is also available at

`https://biosolveit.de/products/#FlexS`.

Complementary tools, especially the graphical platforms SeeSAR and infiniSee, can be obtained from the BioSolveIT website (`https://biosolveit.com`).

References

[1] Christian Lemmen, Claus Hiller, and Thomas Lengauer. Rigfit: A new approach to superimposing ligand molecules. *Journal of Computer-Aided Molecular Design*, 12:491–502, 1998.

[2] Christian Lemmen and Thomas Lengauer. Time-efficient flexible superposition of medium-sized molecules. *Journal of Computer-Aided Molecular Design*, 11:357–368, 1997.

[3] Christian Lemmen, Thomas Lengauer, and Gerhard Klebe. Flexs: A method for fast flexible ligand superposition. *Journal of Medicinal Chemistry*, 41:4502–4520, 11 1998.

We wish you great success and much joy with FlexS!