ExSSO: **Ex**haustive **S**earch for **S**ymmetric **O**ligomer

Structure prediction of symmetric transmembrane oligomers by using NOE distance restraints

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1 About ExSSO

ExSSO is a fast and efficient software for predicting structures of symmetric transmembrane (TM) oligomers. It applies an exhaustive search guided by ambiguous inter-protomer NOEs to find the proper structures of the symmetric oligomer. The user should provide the predetermined protomer structure, inter-protomer NOE restraints and the number of protomers. Then, ExSSO will calculate representative structures with RMSD less than 1.5 Å compared with given NOE distance restraints. These structures can be further refined using the standard MD/SA programs.

In ExSSO, each protomer is treated as a rigid body whose orientation and position relative to the symmetry axis are evaluated. The protomer structure and oligomeric state must be predetermined. ExSSO assigns the Z-axis as the axis of symmetry and samples the orientation of the protomer by performing an Euler rotation around its center-of-mass with Euler angles α, β, and γ. To ensure near complete and uniform conformational sampling, ExSSO uses the following search grid: $\alpha = 0 - 2\pi$, $\Delta\alpha = 5°$; $\beta = 0 - \pi/2$, $\Delta\beta = 5°$; $\gamma = 0 - 2\pi$, $\Delta\gamma = 5°/\sin(\beta)$. Subsequently, the oriented protomer is placed at distance r between the Z-axis and its center-of-mass. By default, the distance r is set to the range $3 - 15$ Å and the step size $\Delta r = 0.5$ Å is used, because these values were found optimal for the sizes of most TM oligomers investigated by NMR. Moreover, the user is given the option to adjust these settings if needed. For each configuration of the protomer, the oligomer structure is constructed by generating symmetric copies of the protomer around the Z-axis using the rotational symmetry operator. Structures with steric clashes, as indicated by inter-protomer distances between Cβ atoms, are not considered. Then each of the oligomer structures is evaluated against the inter-protomer restraints.

2 Input Files & Format

ExSSO requires at least two input files: the protomer structure file and the inter-protomer restraint file (the membrane constraint file or other new implemented constraint files are optional). The protomer structure file contains the atomic coordinates of the protomer in standard PDB format.

The inter-protomer NOE restraints have the following format:

(AA *ResNum1* and ATOM *AtomName1*) (AA *ResNum2* and ATOM *AtomName2*)

where *ResNum* is the residue number in the protomer structure and *AtomName* must be HN, HA, HB, HG, HD, HE, HZ or HH.

The membrane restraint has the following format:

(AA *ResNum1* and *ResNum2*) (AA *ResNum3* and *ResNum4*) *Distance Uncertainty*

where *ResNum1* and *ResNum2* represent the location of one membrane boundary, *ResNum3* and *ResNum4* represent the location of the other membrane boundary, *Distance* is the estimated thickness of lipid bilayer, and *Uncertainty* is the error.

All examples can be found in the ExSSO package (ExSSO/example).

3 How to Use ExSSO

Unpack the ExSSO package to the directory called ExSSO, then change directory

$ cd ExSSO/script

1. Open configure.py and set parameters including protein name ($*proName*), home directory of ExSSO ($*homeDir*), file path of the protomer structure, file path of NOE restraints and the number of protomers, users can also change default parameters, then run this script

$ python configure.py

A file called *param* will be created in $*homeDir*/$*proName*/, which contains all parameters of ExSSO.

2. To check the format of protomer structure file and restraint file, run the following script

$ python check_format.py -p $*homeDir*/$*proName*/param -t pdb

for checking the PDB file only

$ python check_format.py -p $*homeDir*/$*proName*/param -t noe

for checking the NOE restraint file only

$ python check_format.py -p $*homeDir*/$*proName*/param -t mem

for checking the membrane restraint file only

$ python check_format.py -p $*homeDir*/$*proName*/param -t all

for checking all prerequisite input files

3. To search conformations, run the following script

$ python exhaustive_search.py -p $*homeDir*/$*proName*/param

Representative conformations are stored in $*homeDir*/$*proName*/$*proName*.cs

4. To generate the final structures of symmetric oligomer, which can be fed into Xplor-NIH for further refinement, run the following script

$ python get_structure.py -p $*homeDir*/$*proName*/param

All cluster center structures ($*proName*_*.pdb) can be found in $*homeDir*/$*proName*/. The structures are sorted from the best to the worst.