GlycoSHIELD tutorial

Tutorial files include a N-cadherin EC5 domain (EC5.pdb), which natively contains three glycosylation sites at positions 463, 492 and 533. In the tutorial, we will glycosylate the protein with high-mannose N-glycans (Man5) and visualise the span and shielding of the resulting sugar cover. The necessary glycan conformers are located in the GLYCAN_LIBRARY folder and include Man5.pdb (reference file) and Man5_dt1000.xtc (conformer library sampled at 1000ps intervals). Additional files containing different glycans have to be downloaded by users to a local directory and unzipped prior to use.

Execute all commands in the TUTORIAL folder.

The prepared input file (EC5_input) manages the glycan grafting:

```
A 462,463,464 1,2,3 GLYCAN_LIBRARY/Man5.pdb GLYCAN_LIBRARY/Man5_dt1000.xtc A_463.pdb
A_463.xtc
A 491,492,493 1,2,3 GLYCAN_LIBRARY/Man5.pdb GLYCAN_LIBRARY/Man5_dt1000.xtc A_492.pdb
A_492.xtc
```

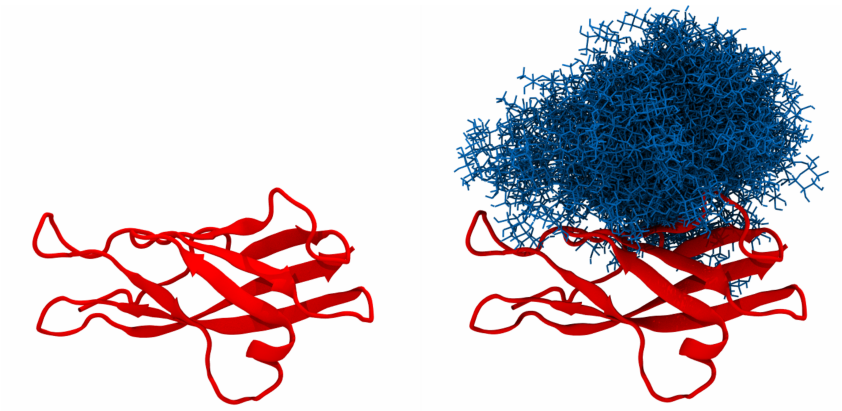
To graft glycans at the selected positions, run:

```
python ../GlycoSHIELD-0.1.py --protpdb EC5.pdb --inputfile EC5_input --threshold 3.5 --mode
CG --shuffle-sugar
```

The program prints out the number of protein conformer (unless protein XTC file was given, it will be always 0) and a number of grafted glycan conformers for each of the three glycosylation sites:

```
0 2619 1172 2569
```

In addition, A_463.pdb,… and A_463.xtc,… files are created, each containing protein structure and a given number of glycan conformers, which can be readily visualised e.g. using VMD:



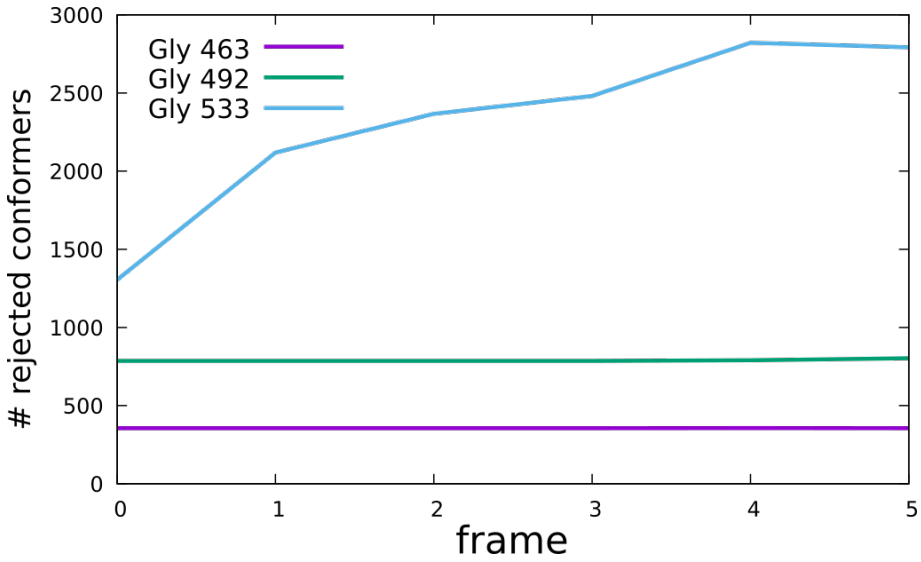
If multiple conformations of the same protein are available, GlycoSHIELD can be used to estimate the entropic cost of geometrically blocking certain glycan conformers in each protein conformer. An artificial trajectory EC4_EC5.xtc with corresponding PDB file EC4_EC5.pdb can be used to visualise this functionality. In each consecutive frame, the angle between the two domains is decreased. To monitor the number of accepted conformations, the following command should be issued:

```
python ../GlycoSHIELD-0.1.py --protpdb EC4_EC5.pdb --protxtc EC4_EC5.xtc --inputfile
EC5_input --threshold 3.5 --mode CG --shuffle-sugar --ignorewarn --dryrun
```

--ignorewarn ensures the grafting continues even if no conformers can be grafted, --dryrun disables production of an output trajectory and instead only displays the number of accepted frames.

Knowing the number of all available frames (3000), the number of rejected frames can be then plotted:

```
0 2645 2214 1695
1 2645 2214 881
2 2645 2214 633
3 2645 2214 519
4 2644 2210 179
5 2645 2197 208
```

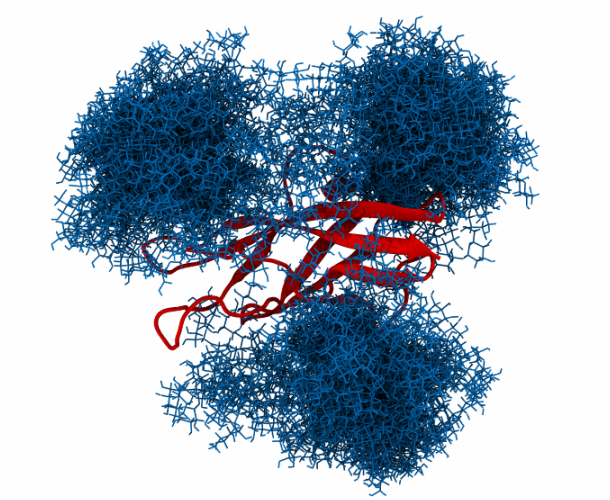


This illustrates that excessive bending of the inter-domain linker results in a rejection of the majority of the conformers and would be thus associated with significant entropic penalty.

In order to generate a single trajectory with all mobile glycans, the number of glycan conformers has to be adjusted to be the same across all glycosylation sites. This can be done using the script “GlycoTRAJ.py”:

```
python ../GlycoTRAJ-0.1.py --maxframe 1172 -outname <merged trajectory> --pdblist
A_463.pdb,A_492.pdb,A_533.pdb --xtclist A_463.xtc,A_492.xtc,A_533.xtc --chainlist A,A,A
--reslist 463,492,533
```

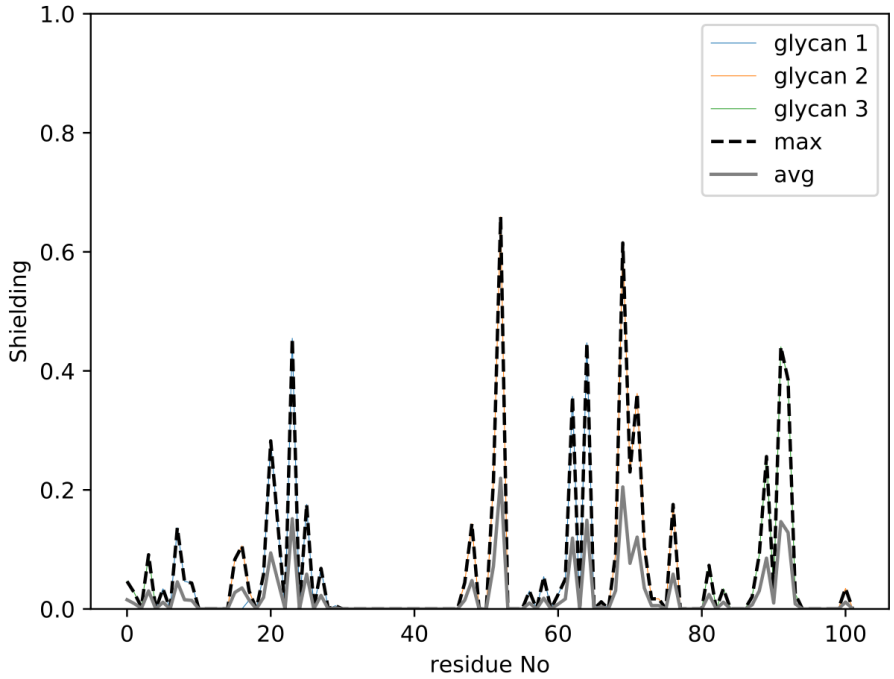
where --maxframe takes the desired number of glycan conformers and --outname is the root name of the output (.xtc will be appended automatically). In this example, 1172 is the largest number of conformers present on all sites.



Trajectories containing multiple conformations of single glycans can be used to calculate a shielding score with “GlycoSASA.py”. Using the example above, the syntax should be:

```
python ../GlycoSASA-0.1.py --pdblist A_463.pdb,A_492.pdb,A_533.pdb --xtclist
A_463.xtc,A_492.xtc,A_533.xtc --probelist 0.14,0.25 --endframe 1172 --plottrace
```

This will analyze 1172 conformers of each glycan to calculate the protein shielding score. As an output, the user obtains a plot of shielding score along the protein sequence:



In addition, a PDB file named “maxResidueSASA_probe_0.14.pdb” is generated, where shielding has been encoded in the column holding the beta factor information. The values 0.14 and 0.25 in the command denote the probe radius used for SASA calculation. In addition, an occupancy value of 1 marks residues accessible in the absence of the glycans, and 0 if a given site is not accessible at all. Such PDB file can then be displayed, e.g. in VMD: