Shc Inhibitors Docking Protocol and Results

February 14, 2021

1 Software

This section briefly describes the primary software used for conducting and preparing ligand docking simulations.

1.1 Rossetta

The Rosetta suite was used to preform the actual ligand docking simulations. Rosetta was downloaded and compiled to a remote cluster where all simulations were ran.

I often accessed the following resources when determining how to run Rosetta for ligand docking.

1. Rosetta ligand docking demo
2. Rosetta Ligand docking with flexible XML protocols
3. Rosetta Documentation

1.2 BioChemicalLibrary (BCL)

BCL was used for generating libraries of conformers (different possible conformations of a ligand) from one specific structure. These conformer libraries would then be given to Rosetta in order to simulate ligand flexibility.

1.3 Open Bable

The Open Babel suite was used for one-off file conversions in cases where `sdf` files needed to be converted to `pdb` or similar operations were required.

1.4 PyMol

PyMol was used to to visualize the results of ligand docking experiments, create images and present results.

1.5 SLURM

All significant compute (namely docking experiments) where ran using the workload manager SLURM. Some programs will not work (RDBC) if other workload managers are used.

1.6 RDBC

Rosetta ligand docking batch job submission control and organizer is a Python command line program I created to help me submit large number of Rosetta docking simulations to the remote cluster's workload manager, SLURM.

2 General workflow

This section describes the general procedure for running docking simulations to access the binding of a specific ligand to a specific protein target.

2.1 Prepare protein structure

First, a ligand free protein structure was repacked using the `ligand_rpkmin.static.linuxgccrelease` program of the Rosetta suite. Repacking was repeated for 100 structures and the lowest energy structure is selected as the docking target.

2.2 Prepare ligand

A file that describes the ligand geometry and properties needs to be generated. Rosetta requires this "params" file for docking any small molecule not already present in the Rosetta database, which in practice are most small molecule. For `RTX ...` drugs this was just a matter of using the `sdf` files as the starting material. If the simulation involved docking a ligand / peptide from an existing co-crystal structure Pymol was used to create a `pdb` file containing only the ligand / peptide. Open Bable was then used to convert the `pdb` file to `sdf` format.

Next, the ligand conformer library and Rosetta `params` files was generated for the to-be-docked ligand. This simulates ligand flexibility during docking. This was usually completed with this Python script which wraps the BCL `molecule:ConformerGenerator` program and the Rosetta `molfile_to_params.py` located in `main/source/scripts/python/public/` of a standard Rosetta installation.

2.3 Prepare RDBC files

I almost always used RDBC to actually run the docking simulations on the remote cluster. RDBC works by using templates of files that would be required for individual jobs and filling them out based on the command line arguments to run many jobs. One of the most important is the Rosetta XML docking protocol which determines exactly what Rosetta does during the simulations. For random docking experiments (where the ligand is positioned at a random position around the protein before docking) I used the random_docker_template.xml.

2.4 Submit jobs with an RDBC command

Once all nessecary files are created the Rosetta docking simulations where submitted to SLURM using RDBC. Below is an example command I used to for randomly docking the NPEYp peptide to the 1OY2 shc structure.

```
python3 ~/software/RDBC/rh.py -l ~/jobs/dock_random_NPEYp/ligand \
-p ~/jobs/dock_random_NPEYp/protein/Shc1-PTB_1OY2_0061.pdb \
-o ~/jobs/dock_random_NPEYp/results \
```

```
-e ~/software/rosetta_bin_linux_2020.08.61146_bundle/\
main/source/bin/rosetta_scripts.static.linuxgccrelease \
-i 2000 \
-op ~/software/RDBC/handler/xml_templates/random_docker.xml \
-b ~/jobs/dock_random_NPEYp/templates/NPEYp.sbatch \
-mi 10
```

- -l: Location of my ligand preparation files produced during the *prepare ligand* step. This included
  - NPEYp_conformers.pdb: Conformer structures generated with BCL.
  - NPEYp.params: Rosetta params file generated by molfile_to_params.py
  - NPEYp.pdb: PDB structure of the NPEYp ligand converted from a provided sdf file.
- -p: Path to target protein. Selected during the *prepare protein structure* step.
- -o: Output path. Where I want the results of simulations to be written to.
- -e: Path to Rosetta scripts exe.
- -i: Number of individual simulations each job should run. This is equivalent to the number of poses Rosetta will produce.
- -op: Path to Rosetta XML protocol file. In this case I used one designed for random docking.
- -b: Path to template `batch` file. This is filled out for each individual job in order to submit many smaller jobs instead of one larger one. This avoids issues if the reasrouces you can use for one job are limited (as was my case).
- -mi: Tells RDBC to submit 10 copies of this job, which allows for simulating more poses when resources for individual jobs are constrained.

Then we just wait for our jobs to complete.

2.5 Aggregate run copies

For random docking that submits multiple copies of the same job, I found it easier to aggregate the results into one large file that is easier to work with for plotting in programs like R.

If RDBC was used to submit such a job, it can also be used for aggregating the results using the `-mai` argument. If my jobs created by the example command had just finished they could be aggregated into one large results tab separated file called `NPEYp.agg.tsv` with the command below.

```
RDBC/rh.py -o /home/ethollem/jobs/dock_random_NPEYp/results -mia NPEYp.agg.tsv
```

Each row in this file represented one completed docking simulation. Simulations can be uniquely identified by comparing both the `description` and the `iter_id columns`.

2.6 Add additional data to aggregate score file

In addition to the metrics produced by Rosetta during the docking simulations we are also interested in having some additional data points listed below.

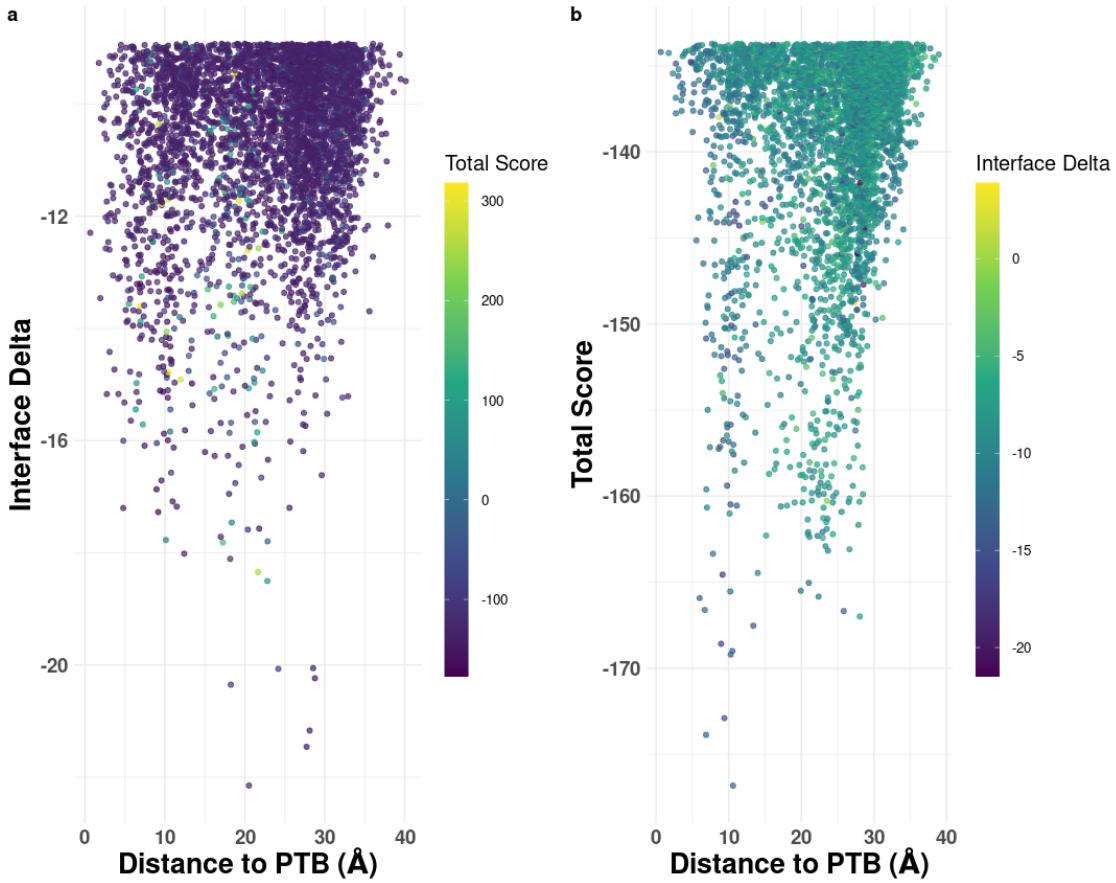
1. Average ligand position: An easy to calculate metric that describes the general location of the ligand in space. Will include three columns, one for each coordinate (x, y, z).
2. Path to pdb file: This is to make viewing the pose easier later on. This will be a column that contains the path to the pdb file representing the final results of the docking simulation. It should be noted that this path will be specific to one machine.

3. Distance to PTB domain. Using the average ligand position we can calculate an approximate euclidean distance to the PTB domain. This value is unsigned.

These data points can be added to aggregated score files using the extend_agg_file.r R script, namely the `extend_agg_file` function contained therein. This function will produce a `RDS` file that can then be opened using the R function `readRDS`.

2.7 Optionally create "energy well" plots

While not strictly required for the analysis you can use the energy_well.r R script in order to create plots that compare distance to the PTB domain (or whatever location is specified when running extend_agg_file.r) to total score and interface delta X of all, or a subset of the simulations. An example plot selecting for the top 15% of results is shown below.



2.8 Identify best poses

With all the results of the docking simulations aggregated into one file, they can be reviewed using any program capable of reading large deliminated text files (I will be using R for the rest of this document).

Two primary metrics where used to access to docking quality of a specific pose.

1. `total_score`: A measure of the overall stability of the protein-ligand complex. Lower values indicate increased stability.

2. `interface_delta_X`: The difference in protein structure stability with and without the ligand in complex. Lower values indicate the ligand has a greater stabilizing effect on the protein structure upon binding and therefore potentially higher affinity.

In order to balance out the two metrics I also assessed poses using a combined metric which considered both interface delta X and total score. This is because I saw a non-zero number of cases where one metric would be very low and the other very high or vice versa.

$$s = 2\frac{s_t - min(s_t)}{max(s_t) - min(s_t)} - 1 + 2\frac{i_\Delta - min(i_\Delta)}{max(i_\Delta) - min(i_\Delta)} - 1$$

Where

- s_t = total score
- i_Δ = interface delta X

This normalizes both total score and interface delta x between -1 and 1 and then adds them together. Poses with both low interface delta X and total score will be ranked higher.

I haven't really seen a combined metric like this used very often, or at least not explicitly in the papers I looked through so I mainly used it as an indicator to access which primary metric might make more sense to use.

3 Results

3.1 Included data

This document is primary housed at a GitHub repository which you can access at this link. The repo also contains many data files that are discussed in part below. Within the `data` directory you will find a directory for each ligand discussed below. The structure of each ligand directory will look something like:

```
.
  best_poses_pdbs
      combined_metric
      interface_delta_x
      total_score
  best_poses_pymol
      interface_delta_x
      total_score
  best_poses_tables
```

- best_poses_pdbs: Contains `pdb` files of the best scoring poses, accessed by multiple metrics.
- best_poses_pymol: Contains PyMol session files of the `pdb` files stored in `best_poses_pdbs`.
- best_poses_tables: `csv` files that were used to generate the tables in this section.

3.2 Docking RTX60933293

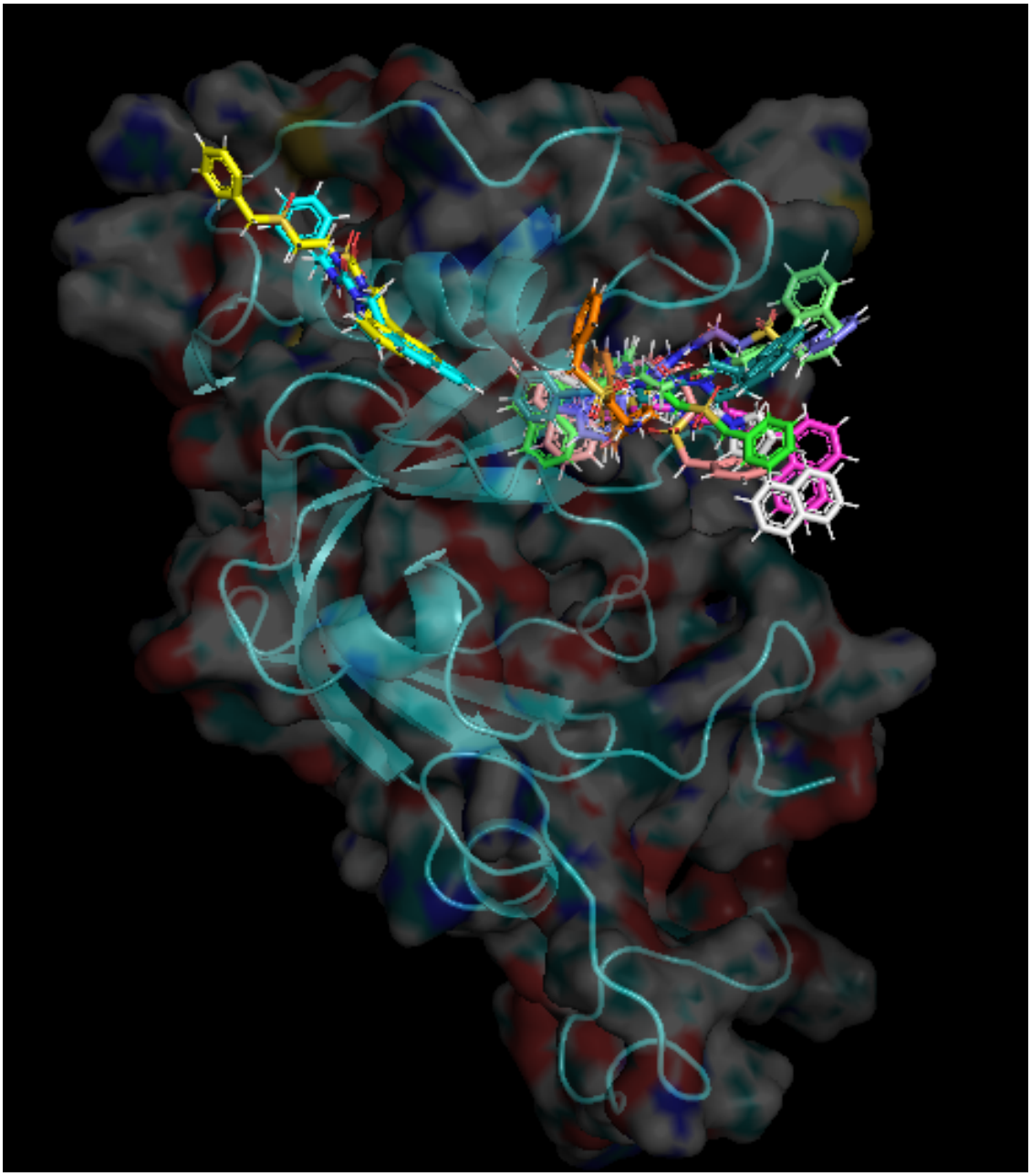
RTX60933293 was docked into a repacked version of `1OY2` (`Shc1-PTB_1OY2_0061.pdb`) using a library of 100 possible conformers and randomized starting positions. 27847 different poses were scored.

3.2.1 Best poses by total score

Filename	Total Score
Shc1-PTB_1OY2_0061_RTX60933293_3913.pdb	-176.816
Shc1-PTB_1OY2_0061_RTX60933293_2879.pdb	-173.871
Shc1-PTB_1OY2_0061_RTX60933293_0313.pdb	-172.901
Shc1-PTB_1OY2_0061_RTX60933293_3287.pdb	-169.206
Shc1-PTB_1OY2_0061_RTX60933293_3917.pdb	-169.001
Shc1-PTB_1OY2_0061_RTX60933293_2406.pdb	-168.58
Shc1-PTB_1OY2_0061_RTX60933293_3818.pdb	-167.529
Shc1-PTB_1OY2_0061_RTX60933293_1493.pdb	-166.992
Shc1-PTB_1OY2_0061_RTX60933293_0693.pdb	-166.676
Shc1-PTB_1OY2_0061_RTX60933293_1167.pdb	-166.612

Link to Pymol Session

Eight of the top ten ligands localize in a “hole” near residue 76 when ranked by total score.



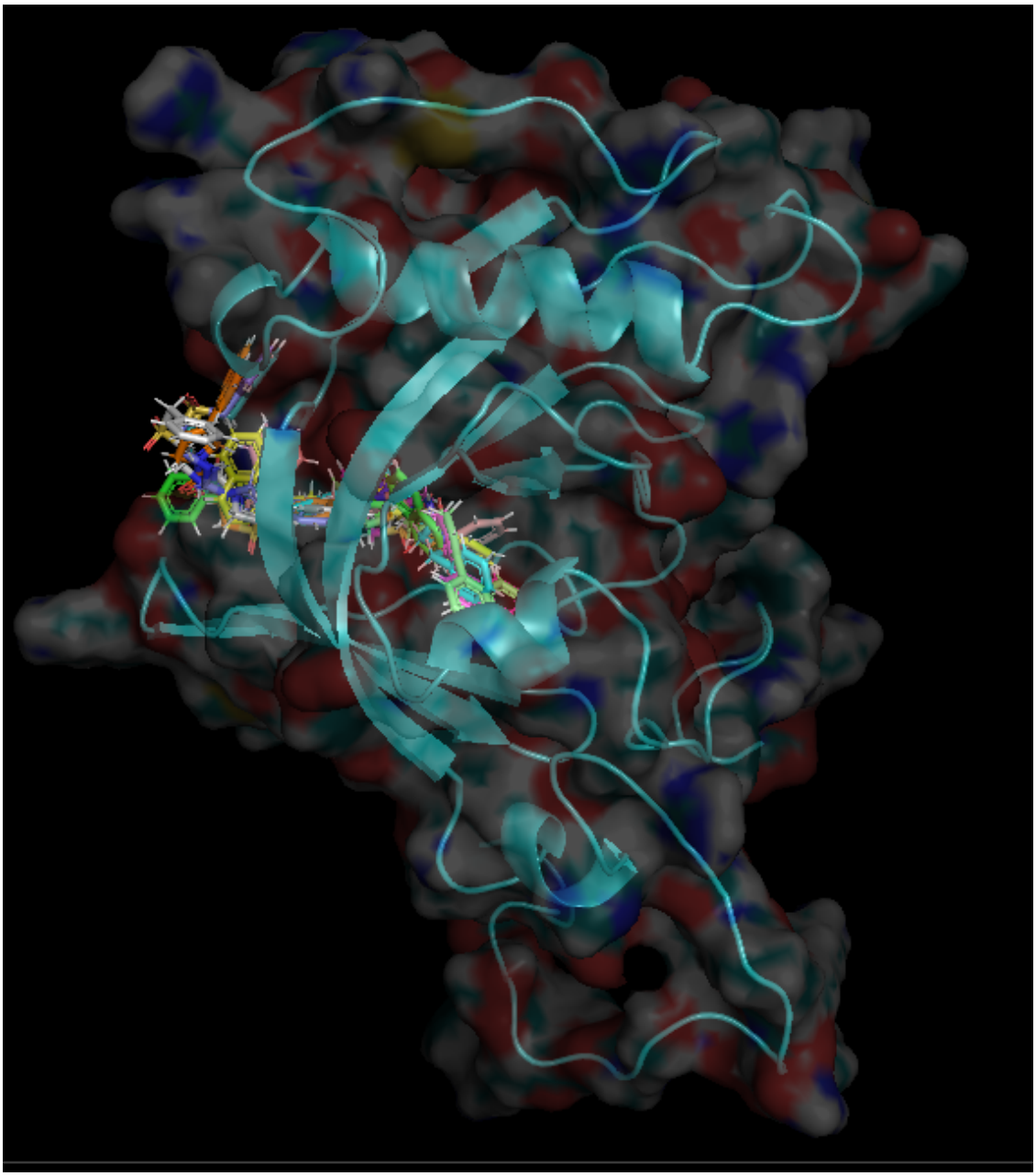
3.2.2 Best poses by interface delta X

Filename	Interface Delta X
Shc1-PTB_1OY2_0061_RTX60933293_0388.pdb	-22.15
Shc1-PTB_1OY2_0061_RTX60933293_3583.pdb	-21.459
Shc1-PTB_1OY2_0061_RTX60933293_1898.pdb	-21.169
Shc1-PTB_1OY2_0061_RTX60933293_2943.pdb	-20.351
Shc1-PTB_1OY2_0061_RTX60933293_2844.pdb	-20.236
Shc1-PTB_1OY2_0061_RTX60933293_0260.pdb	-20.069
Shc1-PTB_1OY2_0061_RTX60933293_1709.pdb	-20.056
Shc1-PTB_1OY2_0061_RTX60933293_0925.pdb	-18.502

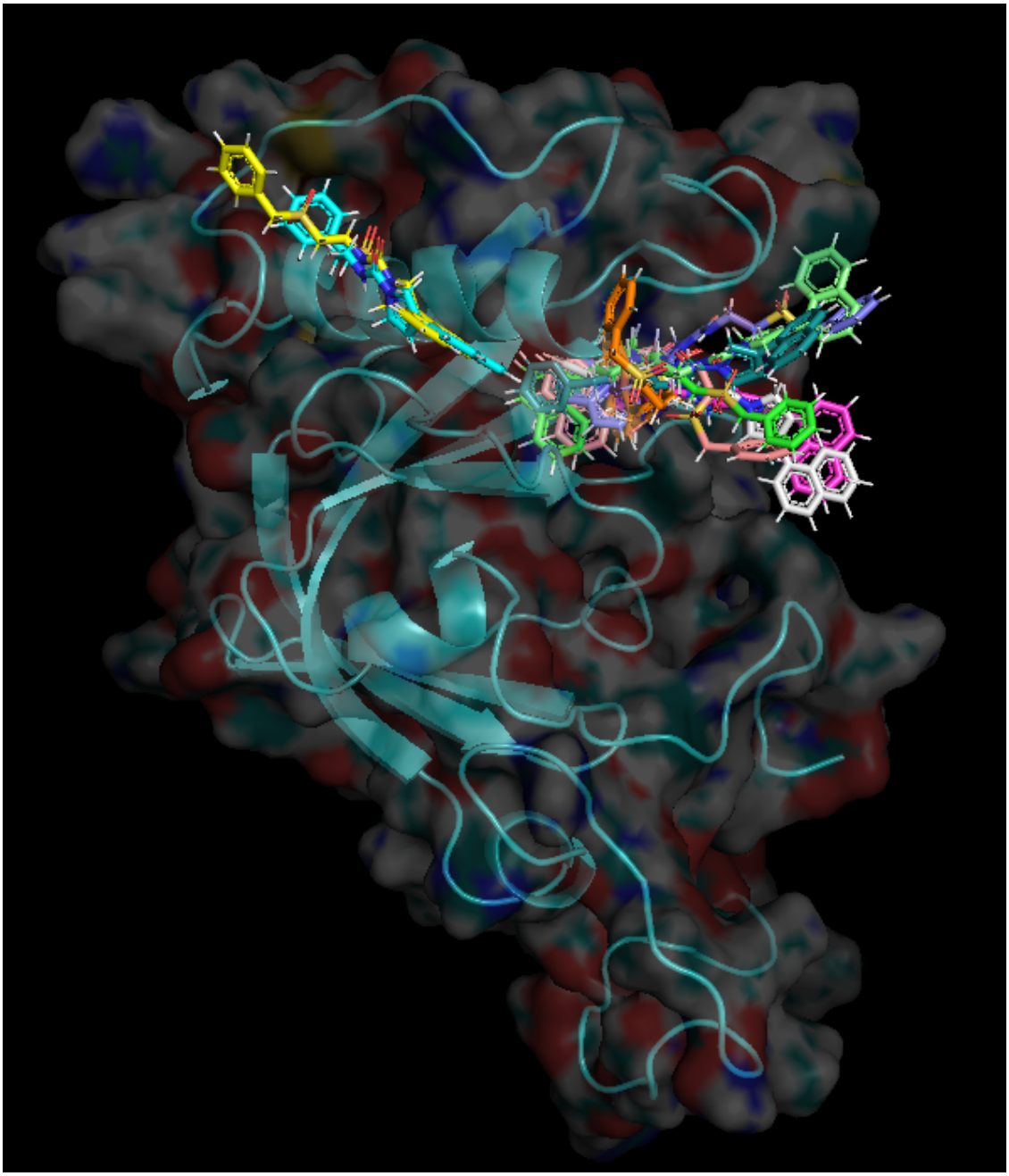
Filename	Interface Delta X
Shc1-PTB_1OY2_0061_RTX60933293_1029.pdb	-18.345
Shc1-PTB_1OY2_0061_RTX60933293_0839.pdb	-18.107

Link to Pymol Session

When ranking poses by interface delta X, ligands cluster to a pocket on the other side of the protein, in closer proximity to the beta sheet.



However, when using the combined metric, all of these poses disappear from the top 10, indicating while the interface delta X was low, the total score was high.



3.3 RTX73145433

RTX73145433 was docked into a repacked version of `1OY2` (`Shc1-PTB_1OY2_0061.pdb`) using a library of 100 possible conformers and randomized starting positions. 39561 different poses were scored.

3.3.1 Best poses by total score

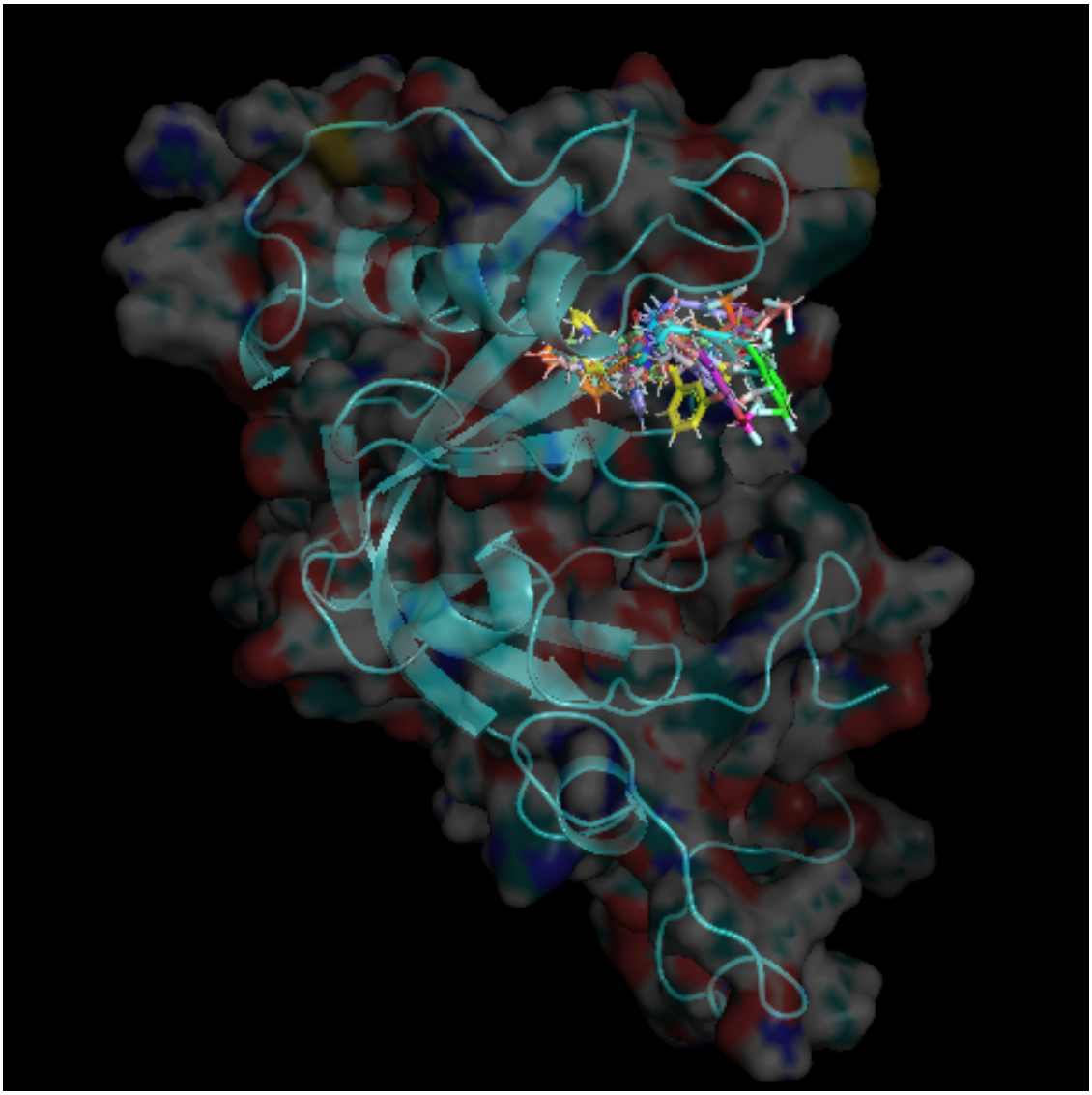
Filepath	Total Score
Shc1-PTB_1OY2_0061_RTX73145433_2004.pdb	-181.271

Filepath	Total Score
Shc1-PTB_1OY2_0061_RTX73145433_1670.pdb	-175.014
Shc1-PTB_1OY2_0061_RTX73145433_2468.pdb	-174.888
Shc1-PTB_1OY2_0061_RTX73145433_3451.pdb	-174.707
Shc1-PTB_1OY2_0061_RTX73145433_0529.pdb	-174.689
Shc1-PTB_1OY2_0061_RTX73145433_1889.pdb	-173.494
Shc1-PTB_1OY2_0061_RTX73145433_1610.pdb	-172.676
Shc1-PTB_1OY2_0061_RTX73145433_0417.pdb	-172.376
Shc1-PTB_1OY2_0061_RTX73145433_1412.pdb	-171.933
Shc1-PTB_1OY2_0061_RTX73145433_3579.pdb	-171.669

The csv version of this table is available from this link

Link to Pymol session

In general, when accessing ligands by total score, there is extremely strong preference for a “hole” hear residue 76.



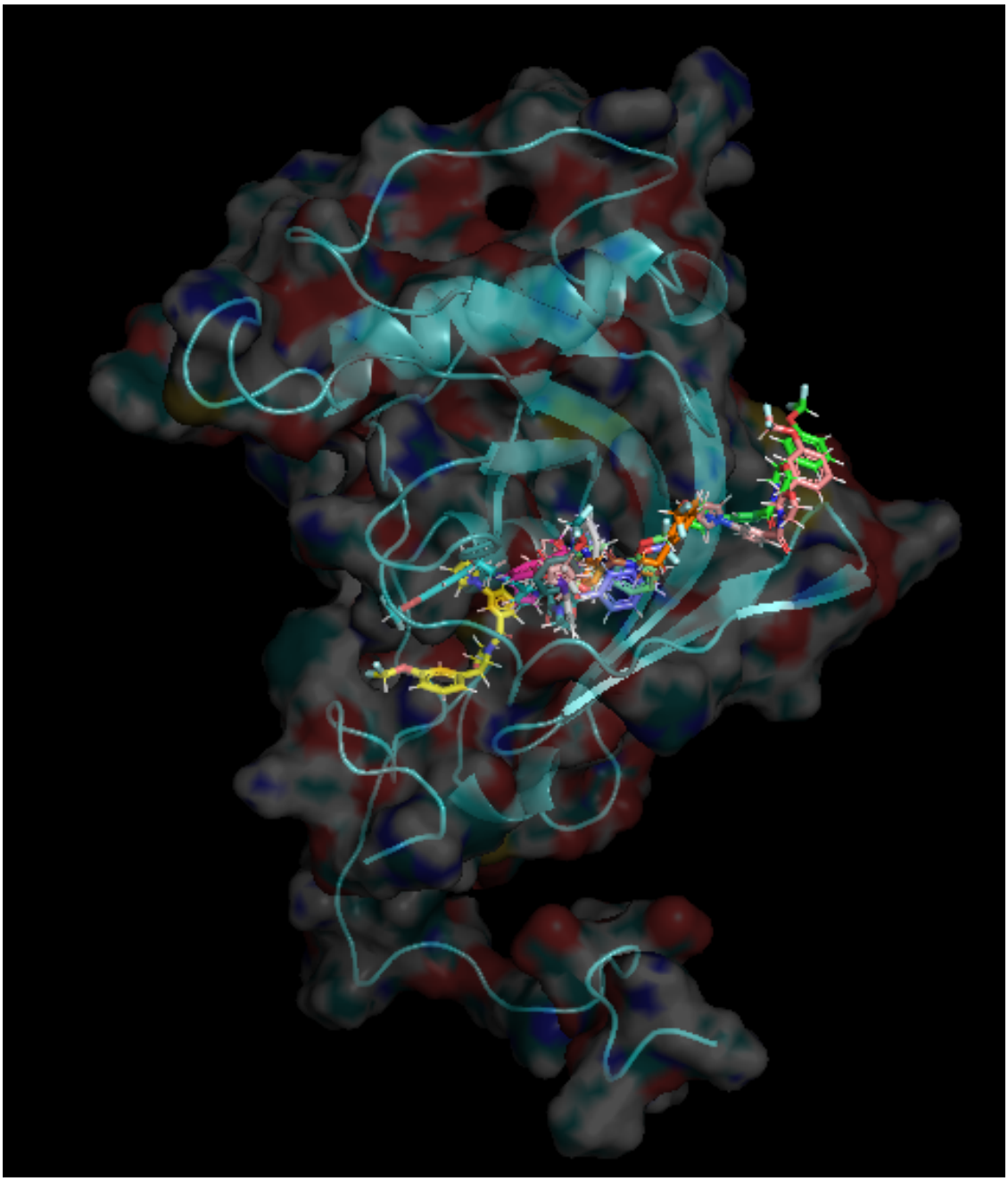
3.3.2 Best poses by interface delta x

Filename	Interface Delta X
Shc1-PTB_1OY2_0061_RTX73145433_2082.pdb	-24.12
Shc1-PTB_1OY2_0061_RTX73145433_2480.pdb	-23.709
Shc1-PTB_1OY2_0061_RTX73145433_2282.pdb	-23.628
Shc1-PTB_1OY2_0061_RTX73145433_2123.pdb	-23.275
Shc1-PTB_1OY2_0061_RTX73145433_3408.pdb	-23.015
Shc1-PTB_1OY2_0061_RTX73145433_0811.pdb	-22.881
Shc1-PTB_1OY2_0061_RTX73145433_1380.pdb	-22.648
Shc1-PTB_1OY2_0061_RTX73145433_2358.pdb	-22.411
Shc1-PTB_1OY2_0061_RTX73145433_2155.pdb	-22.266
Shc1-PTB_1OY2_0061_RTX73145433_0099.pdb	-22.186

The csv version of this table is available from this link

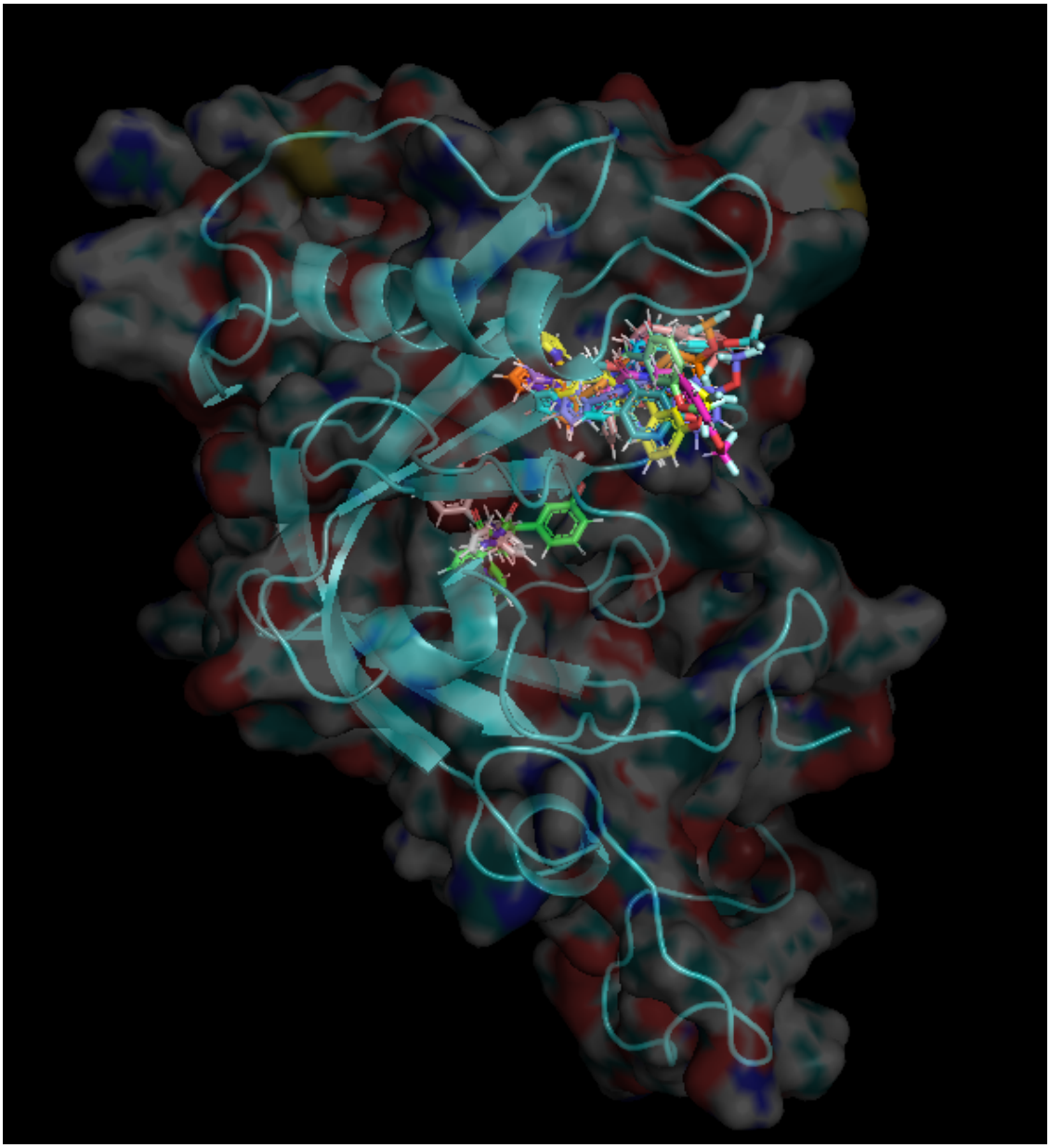
Link to Pymol session

When accessing the best poses by lowest interface delta X ligands tend to cluster on the opposite side of Shc (comapred to clustering as measured by total score)



However, some of these poses have very high total scores including some with positive values, while none of the best ligands as ranked by total score had a positive interface delta X.

When accessing using the combined metric, ligands generally look like those scored using only total score. This is shown in the figure below.



3.4 NPEYp Docking

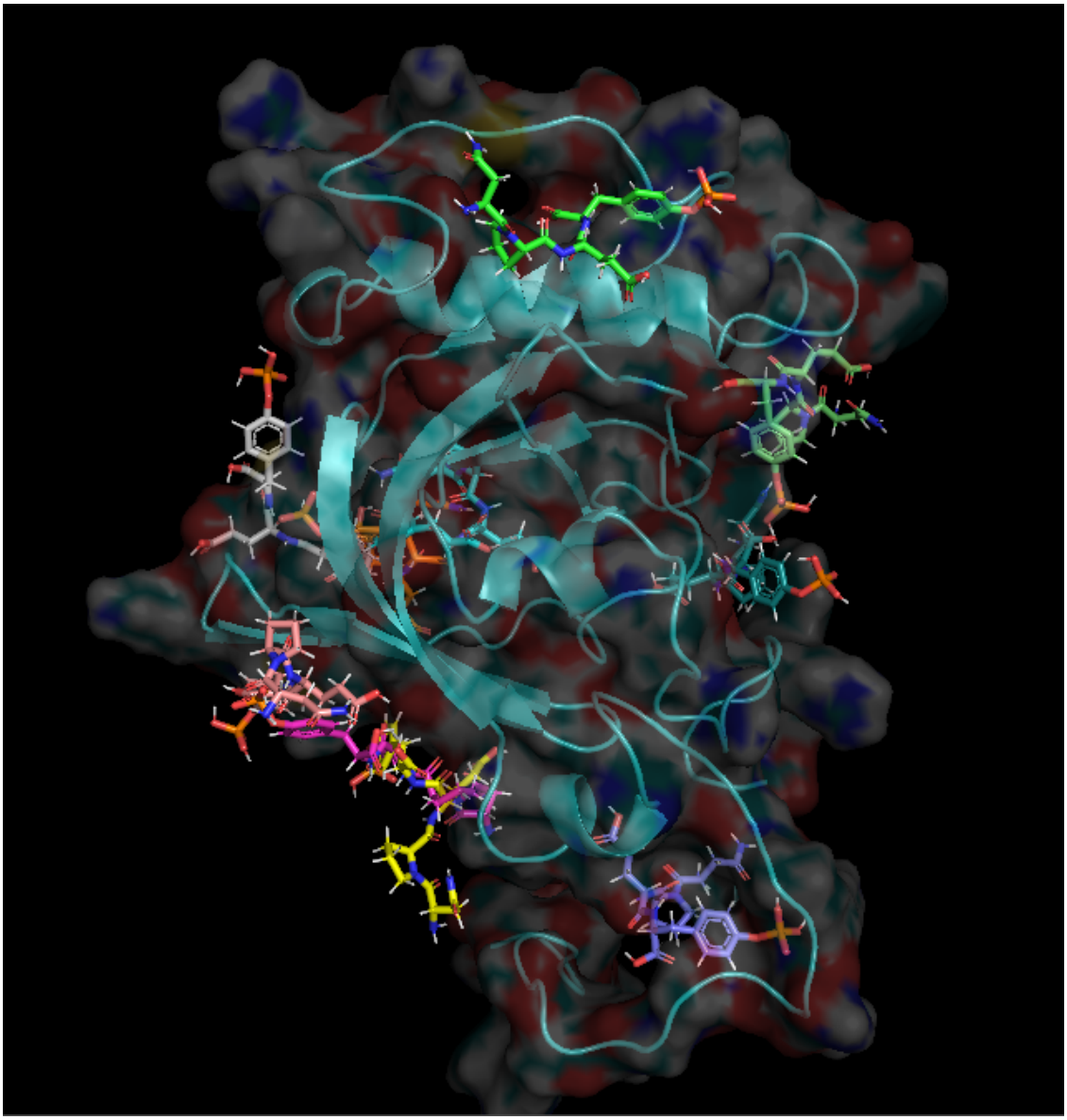
The NPEYp region of the insulin receptor was docked into the 1OY2 receptor using a random starting position and a library of 100 most likely NPEYp conformers generated using BCL. A total of 17695 simulations where completed. The purpose of these simulations was to try and determine possible locations for NPEYp binding and use these locations to access if the Shc inhibiting drugs may be acting competitively or allosterically.

3.4.1 Best poses by total score

Filename	Total Score
Shc1-PTB_1OY2_0061_NPEYp_0537.pdb	-167.35

Filename	Total Score
Shc1-PTB_1OY2_0061_NPEYp_1446.pdb	-167.051
Shc1-PTB_1OY2_0061_NPEYp_0157.pdb	-164.652
Shc1-PTB_1OY2_0061_NPEYp_0269.pdb	-164.631
Shc1-PTB_1OY2_0061_NPEYp_1213.pdb	-164.392
Shc1-PTB_1OY2_0061_NPEYp_0630.pdb	-164.072
Shc1-PTB_1OY2_0061_NPEYp_0753.pdb	-163.858
Shc1-PTB_1OY2_0061_NPEYp_0321.pdb	-163.757
Shc1-PTB_1OY2_0061_NPEYp_0004.pdb	-163.145
Shc1-PTB_1OY2_0061_NPEYp_0834.pdb	-162.682

Link to PyMol session

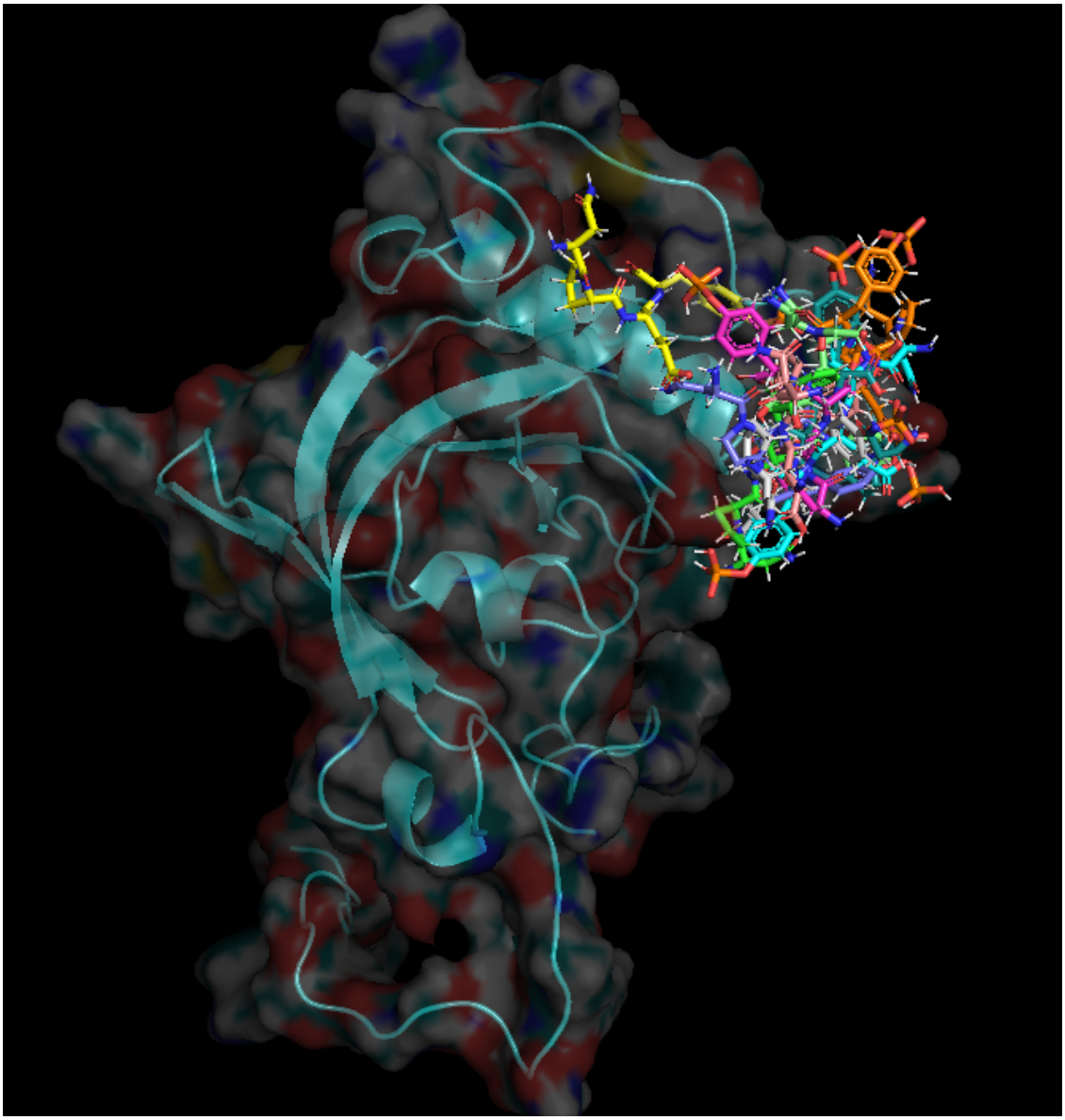


When measuring only by total score, NPEYp top 10 poses where some of the least selective.

Molecules can be seen all around the protein. The interface delta for the poses was reasonable considering ranking was done by total score (`-4.281 -6.266 -7.734 -3.400 -6.974 -5.512 -7.841 -4.556 -5.864 -2.591`). This could suggest that the binding location of NPEYp is very sensitive to the exact conformation of the peptide.

3.4.2 Best poses by interface delta x

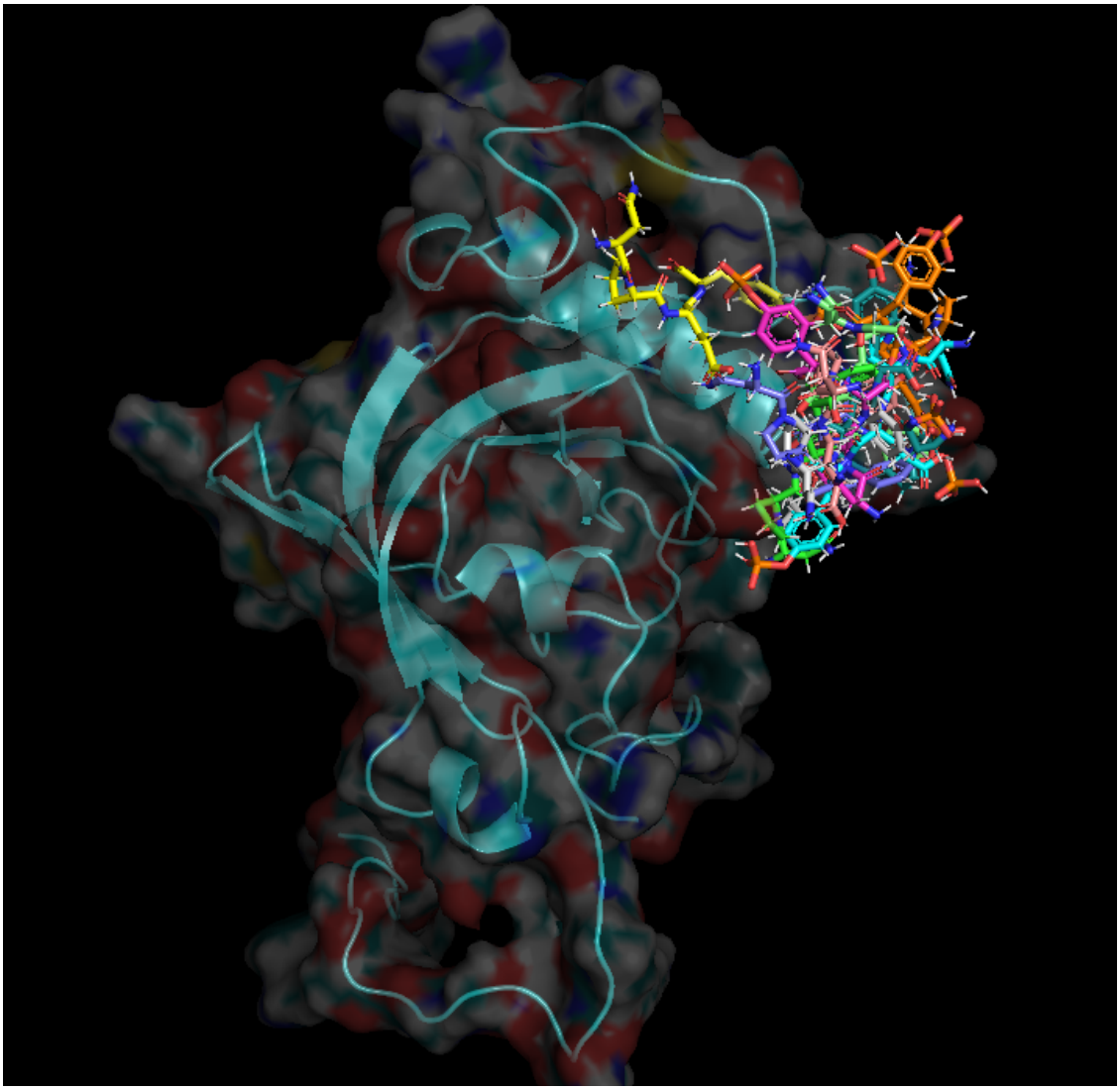
Filename	Interface Delta X
Shc1-PTB_1OY2_0061_NPEYp_1460.pdb	-11.326
Shc1-PTB_1OY2_0061_NPEYp_0303.pdb	-10.987
Shc1-PTB_1OY2_0061_NPEYp_1588.pdb	-10.977
Shc1-PTB_1OY2_0061_NPEYp_1750.pdb	-10.953
Shc1-PTB_1OY2_0061_NPEYp_1372.pdb	-10.686
Shc1-PTB_1OY2_0061_NPEYp_1037.pdb	-10.4
Shc1-PTB_1OY2_0061_NPEYp_0480.pdb	-10.336
Shc1-PTB_1OY2_0061_NPEYp_1109.pdb	-10.22
Shc1-PTB_1OY2_0061_NPEYp_0964.pdb	-10.196
Shc1-PTB_1OY2_0061_NPEYp_0613.pdb	-10.188



In contrast to the results of NPEYp docking when ranking by total score, ranking by interface delta X showed basically opposite results with peptides tightly clustering around the "hole" both RTX drugs had shown preference for at least one metric. This could be indicating that the most stabilizing complexes of Shc-NPEYp occur at this interface.

3.4.3 Best poses by combined metric

Link to PyMol Session



Results when using the combined metric were much more similar to docking poses when ranking by interface delta X. This implies that overall stability of these structures were similar to best structures when ranked only by total score but with better interface delta X values.

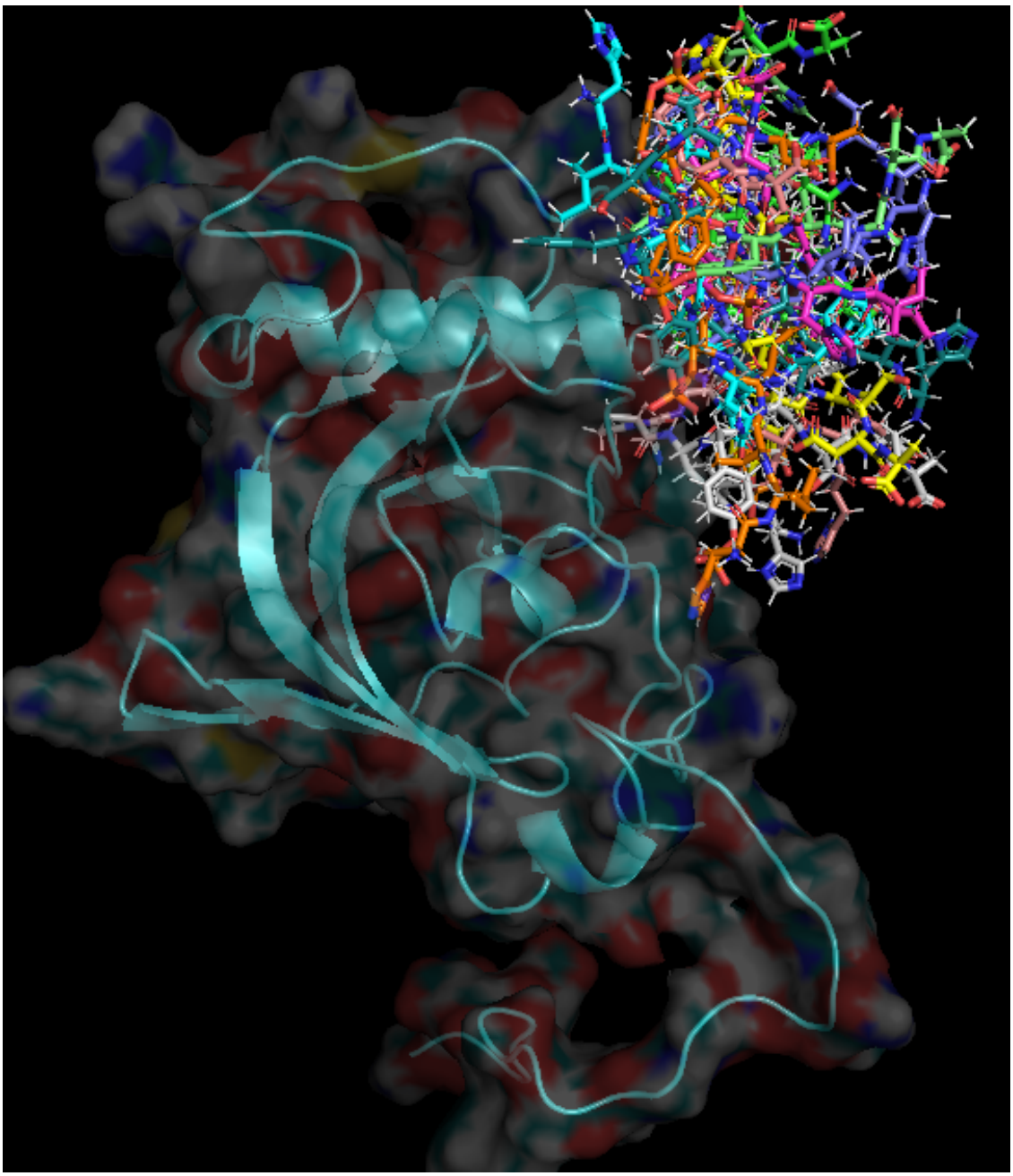
3.5 Trka to 1OY2 docking

The Trka peptide was docked into the same 1OY2 Shc structure used in the simulations above. A total of 12477 simulations where ran and evaluated.

3.5.1 Best poses by total score

Filename	Total Score
Shc1-PTB_1OY2_0061_trka_0007.pdb	-167.695
Shc1-PTB_1OY2_0061_trka_0288.pdb	-166.184
Shc1-PTB_1OY2_0061_trka_0124.pdb	-163.624
Shc1-PTB_1OY2_0061_trka_0200.pdb	-163.604
Shc1-PTB_1OY2_0061_trka_0105.pdb	-163.25

Filename	Total Score
Shc1-PTB_1OY2_0061_trka_0183.pdb	-163.127
Shc1-PTB_1OY2_0061_trka_0003.pdb	-162.872
Shc1-PTB_1OY2_0061_trka_0172.pdb	-162.239
Shc1-PTB_1OY2_0061_trka_0236.pdb	-161.053
Shc1-PTB_1OY2_0061_trka_0094.pdb	-160.684



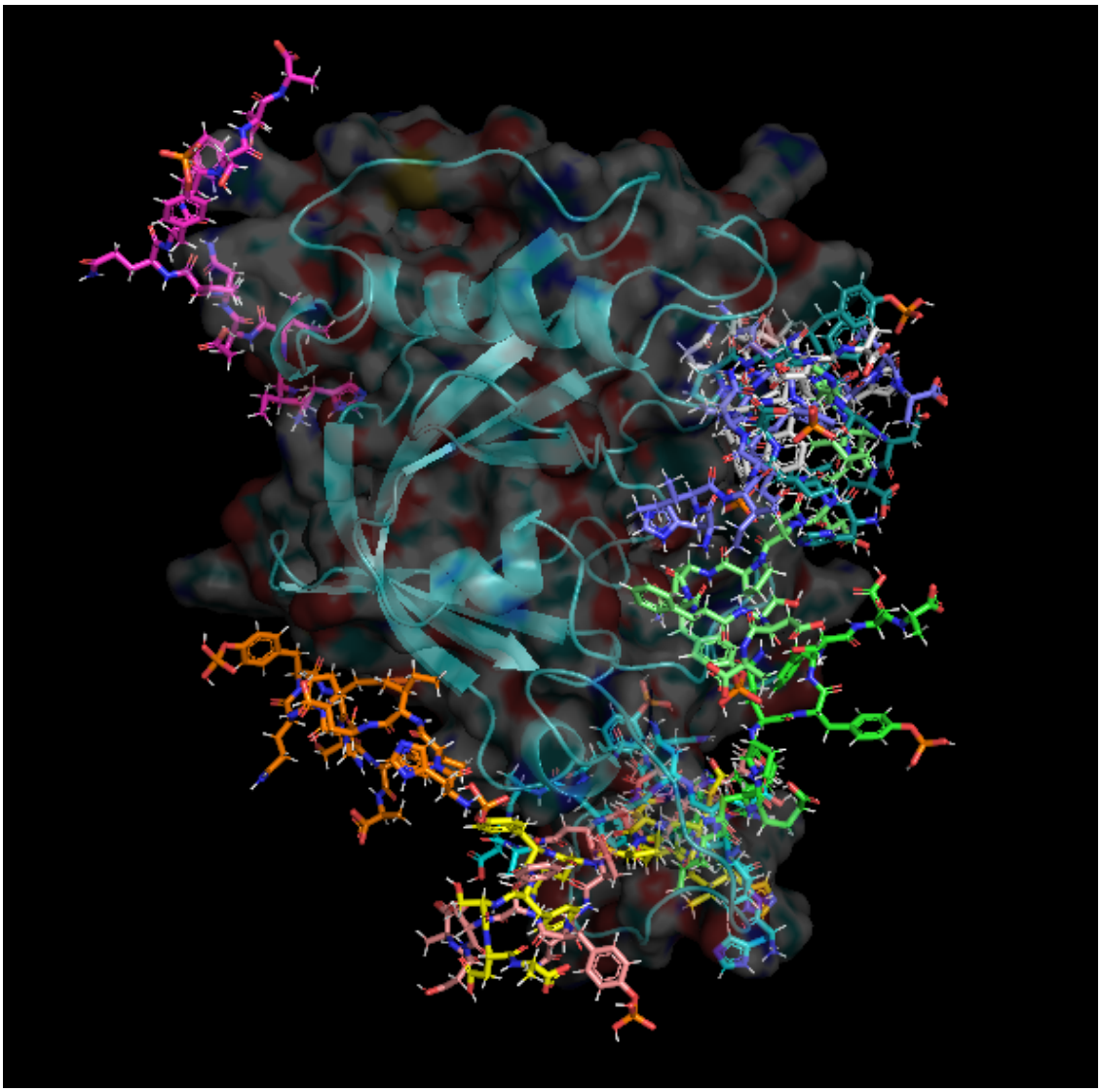
Link to PyMol Session

Docking by total score showed high degree of clustering to the same binding pocket NPEYp favored

when evaluated by total score (and partly interface delta X).

3.5.2 Best poses by interface delta X

Filename	Interface Delta X
Shc1-PTB_1OY2_0061_trka_0051.pdb	-15.562
Shc1-PTB_1OY2_0061_trka_0066.pdb	-13.215
Shc1-PTB_1OY2_0061_trka_0281.pdb	-13.002
Shc1-PTB_1OY2_0061_trka_0303.pdb	-12.916
Shc1-PTB_1OY2_0061_trka_0162.pdb	-12.846
Shc1-PTB_1OY2_0061_trka_0095.pdb	-12.444
Shc1-PTB_1OY2_0061_trka_0249.pdb	-12.301
Shc1-PTB_1OY2_0061_trka_0004.pdb	-12.251
Shc1-PTB_1OY2_0061_trka_0104.pdb	-12.218
Shc1-PTB_1OY2_0061_trka_0087.pdb	-12.165



Link to PyMol Session

3.6 Trka to 1Shc peptide docking

Lastly, the Trka peptide from *Zhou et al* co-crystal structure was removed from the structure and docked back. This was mainly a test to compare the results of docking Trka to 1OY2.

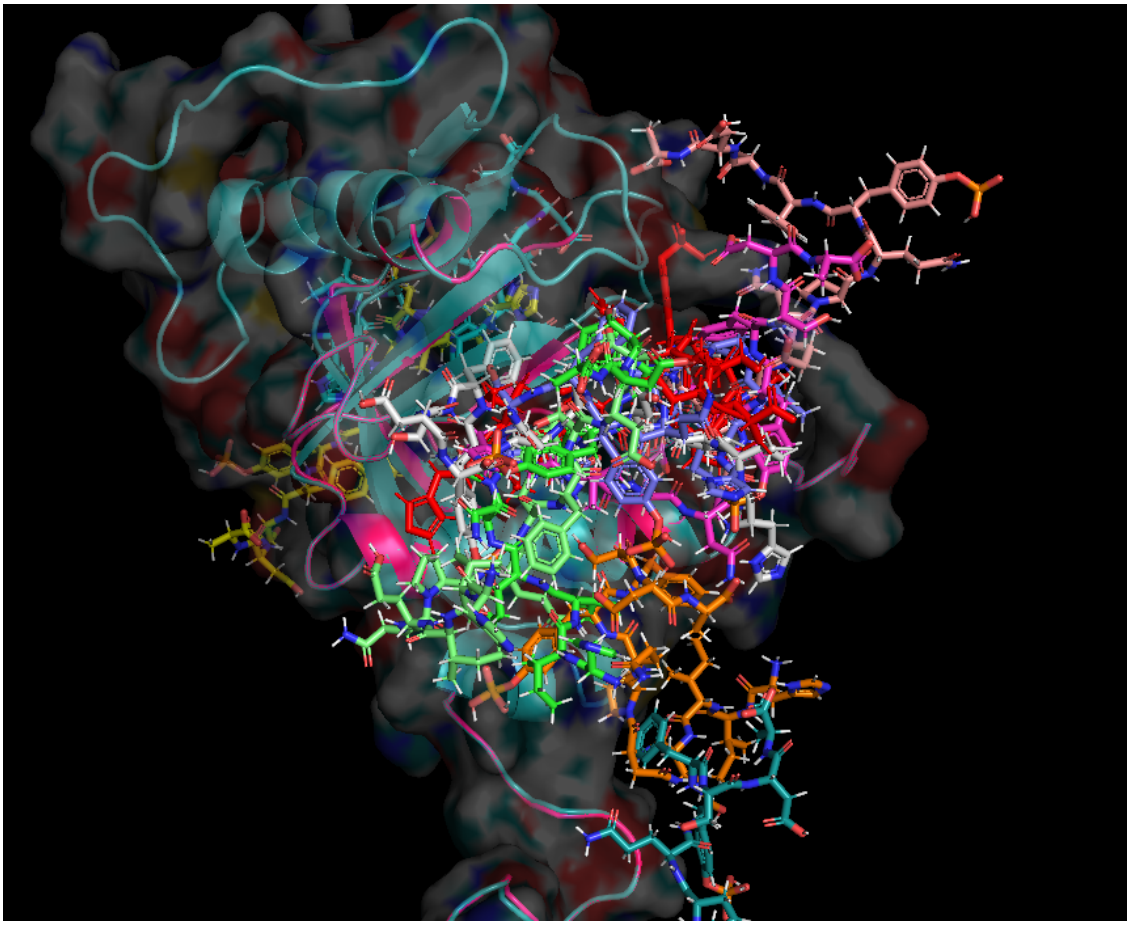
3.6.1 Best poses by total score

Filename	Total Score
1shc_0002_trka_0343.pdb	-126.279
1shc_0002_trka_0795.pdb	-123.926
1shc_0002_trka_0396.pdb	-123.409
1shc_0002_trka_0484.pdb	-118.201
1shc_0002_trka_0266.pdb	-117.033
1shc_0002_trka_1243.pdb	-115.823

Filename	Total Score
1shc_0002_trka_1765.pdb	-115.614
1shc_0002_trka_0665.pdb	-115.468
1shc_0002_trka_0161.pdb	-114.785
1shc_0002_trka_0764.pdb	-114.668

Generally, when measuring by total score the Trka peptide docked in close proximity to the original location (shown in red but hard to see).

Link to PyMol Session



3.6.2 Best poses by interface delta X

Filename	Interface Delta X
1shc_0002_trka_0494.pdb	-14.107
1shc_0002_trka_1649.pdb	-14.042
1shc_0002_trka_1524.pdb	-13.857
1shc_0002_trka_0612.pdb	-13.479
1shc_0002_trka_0258.pdb	-13.388
1shc_0002_trka_0604.pdb	-12.973

Filename	Interface Delta X
1shc_0002_trka_0020.pdb	-12.886
1shc_0002_trka_0985.pdb	-12.842
1shc_0002_trka_0449.pdb	-12.837
1shc_0002_trka_1784.pdb	-12.752

However, this was not the case when measuring by interface delta X, with Rosetta prefering to place Trka on the backside of the protein compared to the co-crystal position.

Link to Pymol Session