



SilcsBio User Guide

Release 2020.2

April 2020

CONTENTS

1	About this document	2
2	Release Notes	3
2.1	Version 2020.2	3
2.2	Version 2020.1	4
2.3	Version 2019.2	4
2.4	Version 2019.1	5
2.5	Version 2018.2	5
2.6	Version 2018.1	5
2.7	Version 2017.2	6
2.8	Version 2017.1	6
3	Graphical User Interface Quickstart	7
3.1	Remote server setup	7
3.2	File and directory selection	9
3.3	SILCS simulation from the GUI	10
3.4	SSFEP simulation from the GUI	20
4	Command Line Interface Quickstart	27
4.1	SILCS simulation from the command line	27
4.2	SSFEP simulation from the command line	28
5	SilcsBio Software Installation	30
5.1	Minimum hardware requirement	30
5.2	Software requirement	31
5.3	Installing the SilcsBio server software	31
5.4	Installing the SilcsBio Graphical User Interface	32
5.5	Installing visualization plugins	33
6	Frequently Asked Questions	36
6.1	1. I installed the software, how do I test if it is correctly installed?	36
6.2	2. I don't have a cluster but I have a GPU workstation. What can I do?	37
6.3	3. I compiled my GROMACS with MPI and my job is not running	37

6.4	4. GROMACS on the head node does not run because the head node and compute node have different operating systems.	38
6.5	5. I get the “error while loading shared libraries: libcudart.so.8.0: cannot open shared object file: No such file or directory” message during my setup.	38
6.6	6. I want to modify the force field and topology files for SILCS simulation	39
6.7	7. I want to visualize FragMaps using MOE	40
7	SILCS: Site Identification by Ligand Competitive Saturation	41
7.1	Background	41
7.2	Running SILCS simulations from the SilcsBio GUI	44
7.3	Running SILCS simulations from the command line interface	45
7.4	SILCS simulation setup with GPCR targets	47
7.5	SILCS simulation setup with halogen probes	48
7.6	Resuming stopped SILCS jobs	49
7.7	Visualizing FragMaps	49
8	SILCS-MC: Ligand Optimization	54
8.1	Background	54
8.2	Running SILCS-MC ligand optimization from the SilcsBio GUI	55
9	SILCS-MC: Docking and Pose Refinement	61
9.1	Background	61
9.2	Running SILCS-MC docking	61
9.3	Running SILCS-MC pose refinement	67
9.4	Best-pose retrieval	68
9.5	User-defined protocols	69
10	SILCS-Pharm: Receptor-Based Pharmacophore Models from FragMaps	75
10.1	Background	75
10.2	Running SILCS-Pharm	76
11	SILCS-Hotspots: Fragment Binding Sites Including Allosteric Sites	85
11.1	Background	85
11.2	Usage and Options	85
11.3	Practical Considerations	90
11.4	Example	91
11.5	Excipient design for biologics	92
12	SSFEP: Single Step Free Energy Perturbation	93
12.1	Background	93
12.2	Usage	94
12.3	Evaluating binding affinity changes	97
13	CGenFF: CHARMM General Force Field	98
13.1	Background	98
13.2	Usage	99

13.3	GROMACS-readable parameters	100
13.4	CGenFF Parameter Optimizer	101
14	References	112
	Bibliography	113

Copyright © 2020 by SilcsBio, LLC

All rights reserved.

No part of this book may be reproduced in any form or by any electronic or mechanical means including information storage and retrieval systems, without permission in writing from SilcsBio, LLC. The only exception is by a reviewer, who may quote short excerpts in a review.

SilcsBio LLC

8 Market Place, Suite 300

Baltimore, MD 21202

info@silcsbio.com

<http://www.silcsbio.com>

ABOUT THIS DOCUMENT

SilcsBio offers a suite of computer-aided drug design tools including: the patented SILCS functional group mapping approach with capabilities for database screening, fragment-based drug design, and lead optimization; and the CGenFF force field parameter assignment engine. This documentation covers how to install and use the software.

RELEASE NOTES

2.1 Version 2020.2

This version adds the following:

- SilcsBio Graphical User Interface (GUI) improvements for file and directory selection allowing for input files to be chosen from remote servers as well as the local computer
- SilcsBio Graphical User Interface (GUI) support for visualization of SILCS-Halogen FragMaps
- A new plug-in for visualizing SILCS FragMaps in MOE
- CGenFF program and parameters update to version 2.4.0

CGenFF coverage has been extended to amide bases and molecules containing boron. The functional groups in these molecules were not previously accessible in CGenFF. The following compounds have been explicitly parameterized and validated, allowing CGenFF to generate parameters for more diverse compounds.

N-methyl acetamide (deprotonated amide); N-ethyl acetamide (deprotonated amide); N-methyl benzamide (deprotonated amide); N-phenyl acetamide (deprotonated amide)

Methyl boronic acid (neutral, -1, -2); Ethyl boronic acid (neutral, -1, -2); Phenyl boronic acid (neutral, -1, -2)

Additionally, 112 naturally-occurring modified nucleotides, especially modified bases with heterocycles not previously covered by CGenFF, have been parametrized. Quantum mechanical calculations on model compounds, including geometries, dipole moments, and interactions with water, provided target data. Selected parameters were validated with extensive molecular dynamics simulations. Details of the parameter optimization and the complete list of nucleotides can be found in [3].

as well as bug fixes and stability improvements.

Validation of the SILCS-HotSpots approach has been published [4].

2.2 Version 2020.1

This version adds the following:

- The SILCS-Hotspots method for identifying all potential ligand binding sites on a protein
- SilcsBio Graphical User Interface (GUI) support for SILCS-Pharm
- SilcsBio Graphical User Interface (GUI) support for SILCS-MC Docking and Pose Generation
- Performance improvements to GCMC-MD
- CGenFF program and parameters update to version 2.3.0

CGenFF version 2.3.0 adds explicit parametrization for the following molecules. In prior versions, functional groups in these molecules were accessible through analogy to related functional groups. Now, explicit parametrization and validation yields more accurate treatment and decreases the associated CGenFF penalty scores.

1H-tetrazole; 5-methyl-1H-tetrazole; 5-ethyl-1H-tetrazole

2-oxetanone; 3-oxetanone

ammonium; dimethylammonium; trimethylammonium (Note: Protonated amine parameters were previously based on methylammonium. While the present explicit parametrization of secondary and tertiary amines leads to smaller penalty scores and improvements in performance, electrostatic interactions will continue to be dominated by the +1 monopole.)

1-butyne; 1-pentyne; 1-hexyne; 1-heptyne; 1-octyne; but-1-ene-3-yne (Note: Alkyne parameters were previously based on ethene and propene. Extension to longer alkynes and the ene/yne combination validates the parameters and leads to improved treatment of the intramolecular interactions.)

CGenFF version 2.3.0 also includes improved halogen-protein interactions. Quantum mechanical calculations on chloro- and bromobenzene with model compounds representative of protein functional groups were used as target data to optimize atom-pair specific Lennard-Jones parameters for selected atoms in the model compounds. Application of the parameters in molecular dynamics simulations of eight ligand-protein systems demonstrated systematic improvement in interaction geometries.

as well as bug fixes and stability improvements.

2.3 Version 2019.2

This version adds the following:

- The SILCS-Pharm method for generating 3D receptor-based pharmacophore models from FragMaps in an automated manner

as well as bug fixes and stability improvements.

2.4 Version 2019.1

This version adds the following:

- The SILCS-Pharm method for generating 3D receptor-based pharamcophore models from FragMaps in an automated manner (early access)

as well as bug fixes and stability improvements.

2.5 Version 2018.2

This version adds the following:

- A new Graphical User Interface (GUI) capable of preparing and launching SILCS, SILCS-MC, and SSFEP jobs on remote computing clusters and analyzing and visualizing the job outputs
- A new CGenFF Parameter Optimizer with functionality for dihedral parameter fitting

as well as bug fixes and stability improvements.

2.6 Version 2018.1

This version adds the following:

- A new Graphical User Interface (GUI) capable of preparing and launching SILCS, SILCS-MC, and SSFEP jobs on remote computing clusters and analyzing and visualizing the job outputs (early access)
- Improved GPCR and other transmembrane protein support for SILCS
- Improved GPCR and other transmembrane protein support for SSFEP
- CGenFF program and parameters update to version 2.2.0

CGenFF version 2.2.0 extends support to a large variety of drug-like molecules to be used routinely in Computer-Aided Drug design projects. Specifically, 1) it improves treatment of halogen bonds by introducing lone-pairs onto the halogen atoms of aromatic systems. 2) Support is extended to four-membered oxetane, glycolruril, and S-P bond found in GTP-gamma like molecules. 3) Improved predictions with partial charge distributions around ammonium ions and primary amines.

- A new CGenFF Parameter Optimizer with functionality for dihedral parameter fitting (early access)

as well as bug fixes and stability improvements.

2.7 Version 2017.2

This version adds the following:

- GPCR support for SILCS
- GPCR support for SSFEP
- CGENFF program and parameter update to version 2.1.0

CGenFF version 2.1.0 extends support to S-P bond found in the GTP-gamma like molecules. Bonded parameters and charge-distribution along the S-P bond were modeled using the CHARMM nucleic acid force-field patches for mono- and di-thio substitutions (top-par_all36_na_reactive_rna.str). Three new atom-types have been added to the CGenFF force-field : SG2P1, SG2P2 to support the mono- and di-thio substitutions, along with OG2S1 to model the terminal oxygen connected to the S-P bond.

as well as bug fixes and stability improvements.

2.8 Version 2017.1

This version is the initial release. This version includes the following packages:

- SILCS command line interface
- SILCS-MC command line interface
- SSFEP command line interface
- CGENFF program and parameter version 2.0.0

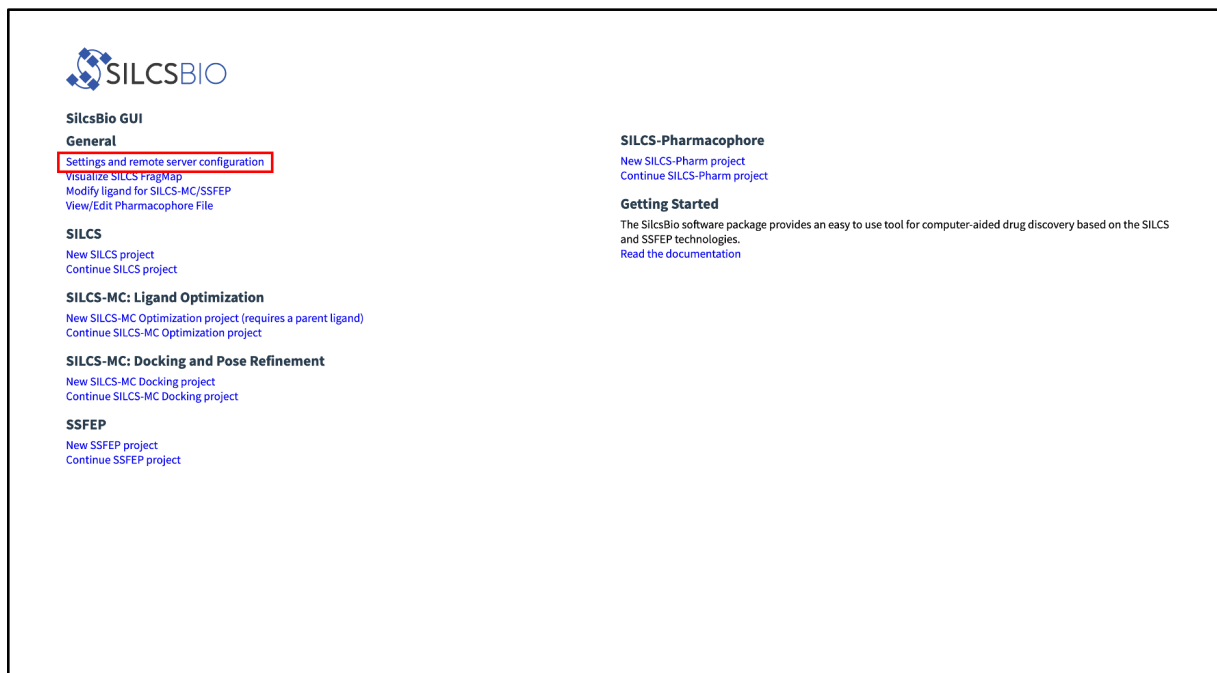
CGenFF version 2.0.0 is the second release of CGenFF, extending support to a larger variety of drug-like molecules to be used routinely in Computer-Aided Drug design projects. Specifically, it improves treatment of halogen bonds, by introducing lone-pairs onto the halogen atoms of aromatic systems. Additionally, support is now extended to four-membered oxetane and glycoluril moieties. This is driven largely by improvements in the newly released version 4.0 of CGenFF force field.

GRAPHICAL USER INTERFACE QUICKSTART

This chapter provides a step-by-step introduction on how to use the SilcsBio Graphical User Interface (GUI).

3.1 Remote server setup

The SilcsBio GUI is designed to work with the server installation of the SilcsBio software. When you launch the GUI, you will be presented with the Home page. From the Home page, select *Settings and remote server configuration*.



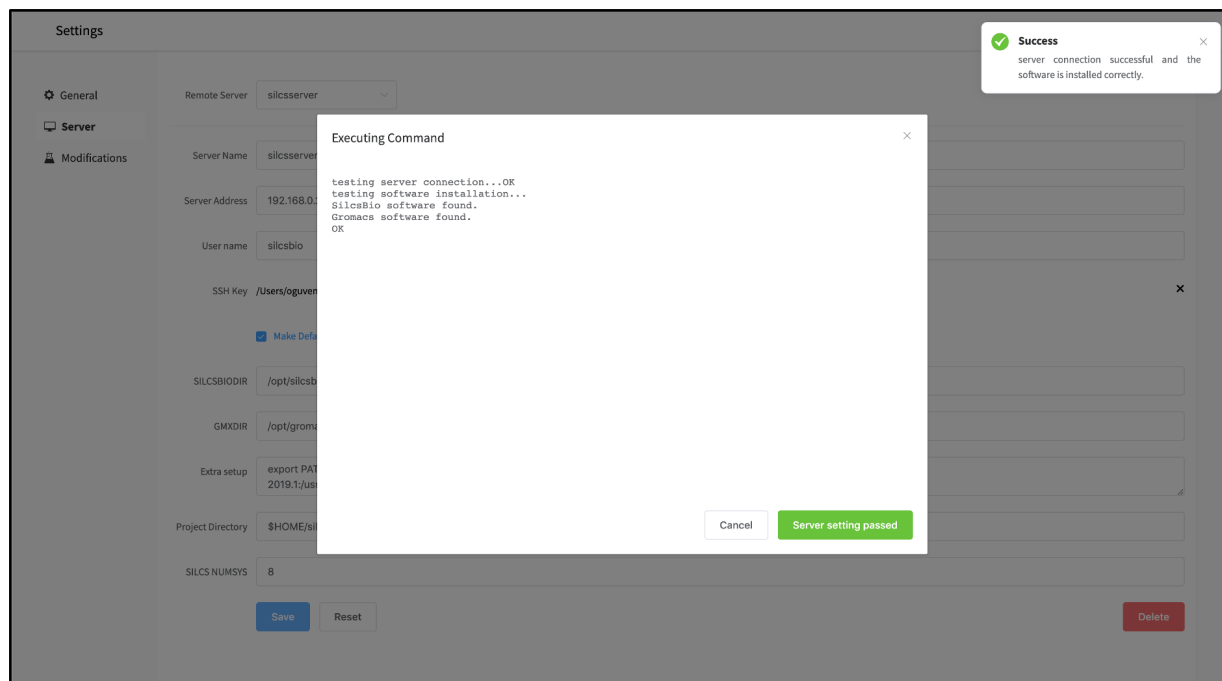
Within the “Settings” page, select *Server* and enter the remote server information, such as Server Address (IP address), Username, and an SSH key to the server. If you do not have an SSH key to

the server, leave it blank. The GUI will ask you the password to the remote server instead. Select the “Make Default Server” checkbox if you would like to set this server as your default server. This server will be selected as a default in other parts of the GUI.

You will also need to enter `SILCSBIODIR` and `GMXDIR` file path values. These should match the values on your server. You may use the “Extra setup” field to pass additional commands to the server, such as exporting environment variables or loading modulefiles. You may use the “Project Directory” field to define the directory on your server where server job input files will be created and server job outputs stored. Typical SILCS simulations produce output files in excess of 100 GB, so please select a project directory file folder with appropriate storage capacity. The “SILCS NUMSYS” field determines how many SILCS jobs are launched for creating FragMaps. Set this to an even integer; we recommend “10” to maximize convergence or “8” to minimize resource use.

The screenshot shows the 'Settings' page for SilcsBio. On the left, there are tabs for 'General', 'Server', and 'Modifications'. The 'Server' tab is selected. The 'Remote Server' dropdown is set to 'silcsserver'. The 'Server Name' field contains 'silcsserver'. The 'Server Address' field contains '192.168.0.26'. The 'User name' field contains 'silcsbio'. The 'SSH Key' field has a 'select file' button and a link 'Where to find a SSH key?'. A red box highlights the 'SSH Key' field with the text 'Either select ssh key or leave blank to use password-based authentication'. Below the 'SSH Key' field is a checkbox labeled 'Make Default Server' which is checked. The 'SILCSBIODIR' field contains '/opt/silcsbio/silcsbio.2020.2.0'. The 'GMXDIR' field contains '/opt/gromacs/gromacs-2019.6/bin'. The 'Extra setup' field contains 'export PATH=/opt/gromacs/gromacs-2019.6/bin:/usr/local/cuda-10.1/bin:/usr/local/cuda-10.1/nightlyCompute-2019.1:/usr/local/sbin:/usr/local/bin:/usr/sbin:/usr/bin:/sbin:/bin:/usr/games:/usr/local/games:/snap/bin'. The 'Project Directory' field contains '\$HOME/silcsbio/projects'. The 'SILCS NUMSYS' field contains 'leave blank for system default'. At the bottom, there are 'Save', 'Reset', and 'Delete' buttons. In the top right corner, there is a 'Go to Home' button with a house icon and a red box around it. Below it, there is a red text link: 'Click here to go back to the landing page'.

Once all information is entered, click the “Save” button. The GUI will save your entries and confirm that you have a working connection to the server.

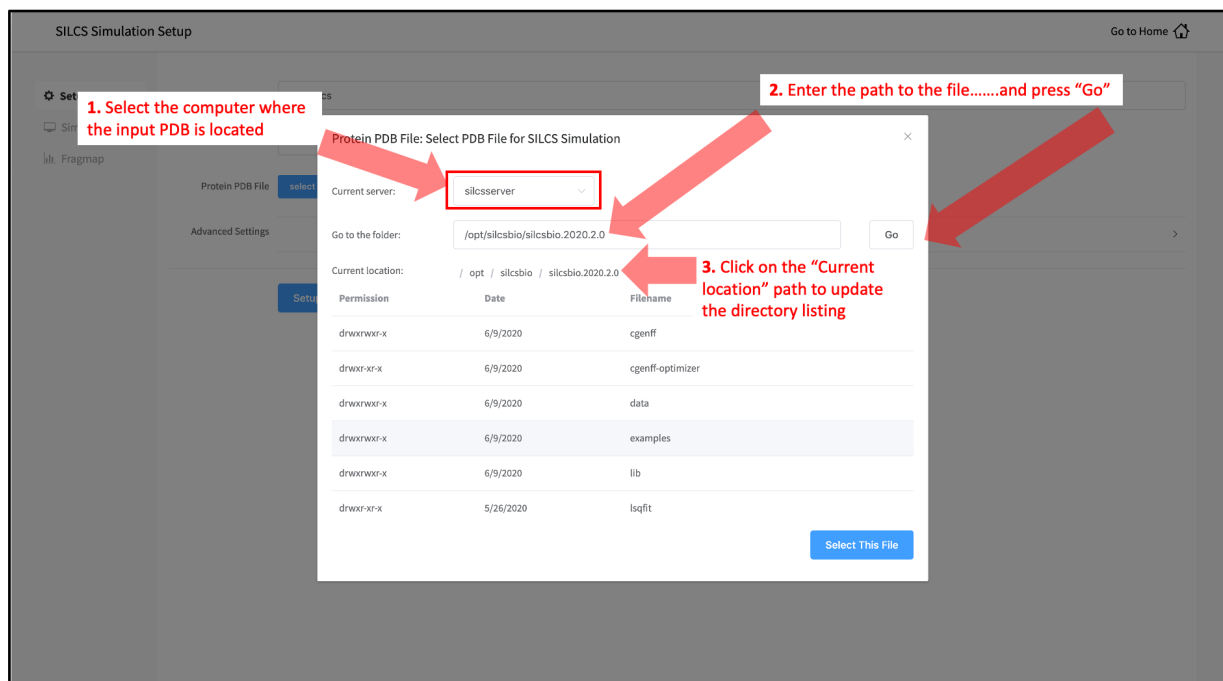


Please contact support@silcsbio.com if you need additional assistance.

3.2 File and directory selection

To run the SilcsBio software, you will need to provide protein and/or ligand input files. The SilcsBio GUI has a standardized user interface that allows you to choose these input files from either the computer on which you are running the GUI or from a remote server you have previously configured. Steps to do this, illustrated in the below figure, are as follows.

1. Choose the “Current server,” which is the physical location of the input file. If you would like to load a file located on the computer on which you are running the GUI, select “localhost”; otherwise, select the remote server name of a server you have previously configured through the *Remote server setup* process.
2. Type in the folder where the file is located using the “Go to the folder” field and then click the associated “Go” button. This will update the “Current location” to this folder. Click on the last portion of the “Current location” path (e.g. “silcsbio.2020.2.0” in “/opt/silcsbio/silcsbio.2020.2.0” in the below example) to update the directory listing located under that path. If you click on any other portion of the “Current location” path, you will be taken to that folder. Double clicking on a folder in the directory listing will move you into that folder.
3. In the directory listing, click on a file to highlight it and click the “Select This File” button at the bottom to finish your file selection.



Follow a similar process to select a directory for those tasks requiring directory selection.

Tip: For those tasks needing FragMaps as inputs, "FragMap Location" needs to be a directory. The SilcsBio GUI assumes a directory path of the form `<basename>/maps/`. Select `<basename>` for your "FragMap Location". The GUI will automatically look for the `maps/` subdirectory and load FragMaps from that subdirectory. It will also automatically load the protein pdb file if one is located in the `<basename>` directory. It may take a few seconds for the GUI to download your input FragMaps if they are not on localhost.

3.3 SILCS simulation from the GUI

To begin a new SILCS project, follow these steps:

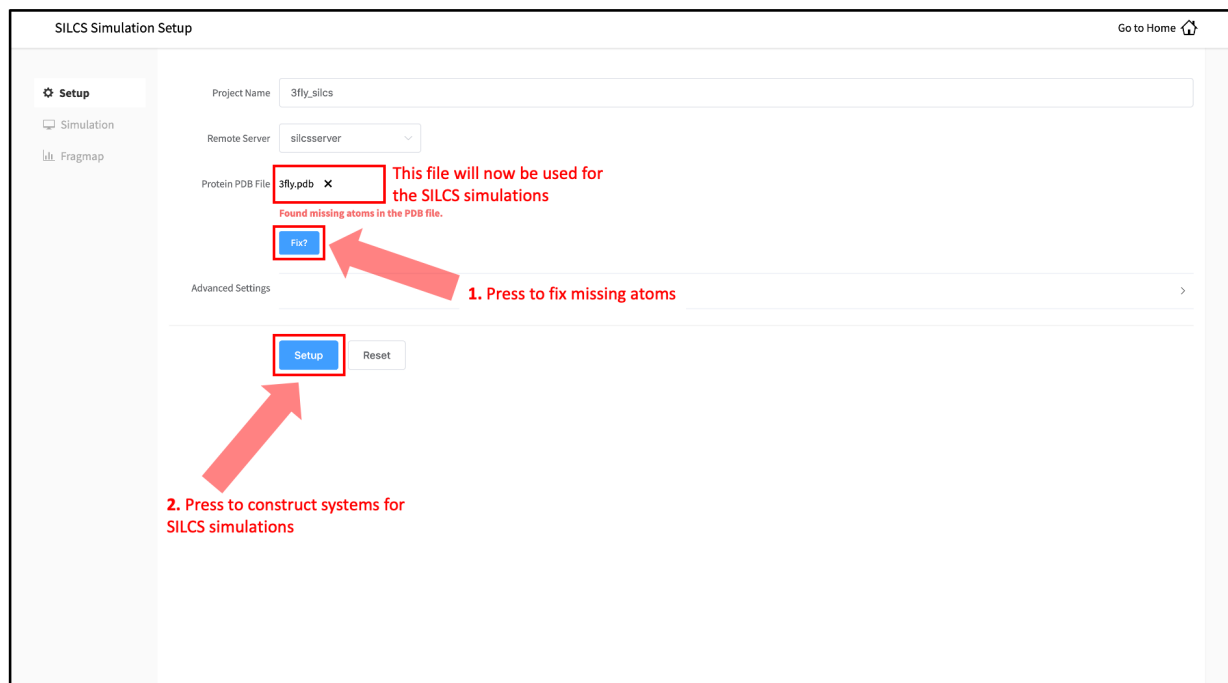
1. Select *New SILCS project* from the Home page.
2. Enter a project name and select the remote server where the SILCS jobs will run. Input and output files from the SILCS jobs will be stored on this server based on your choice of "Project Directory" during the *Remote server setup* process.

The screenshot displays the 'SILCS Simulation Setup' web interface. On the left, a sidebar contains three menu items: 'Setup' (active), 'Simulation', and 'Fragmap'. The main content area is titled 'SILCS Simulation Setup' and includes a 'Go to Home' link in the top right. The 'Setup' section contains the following elements:

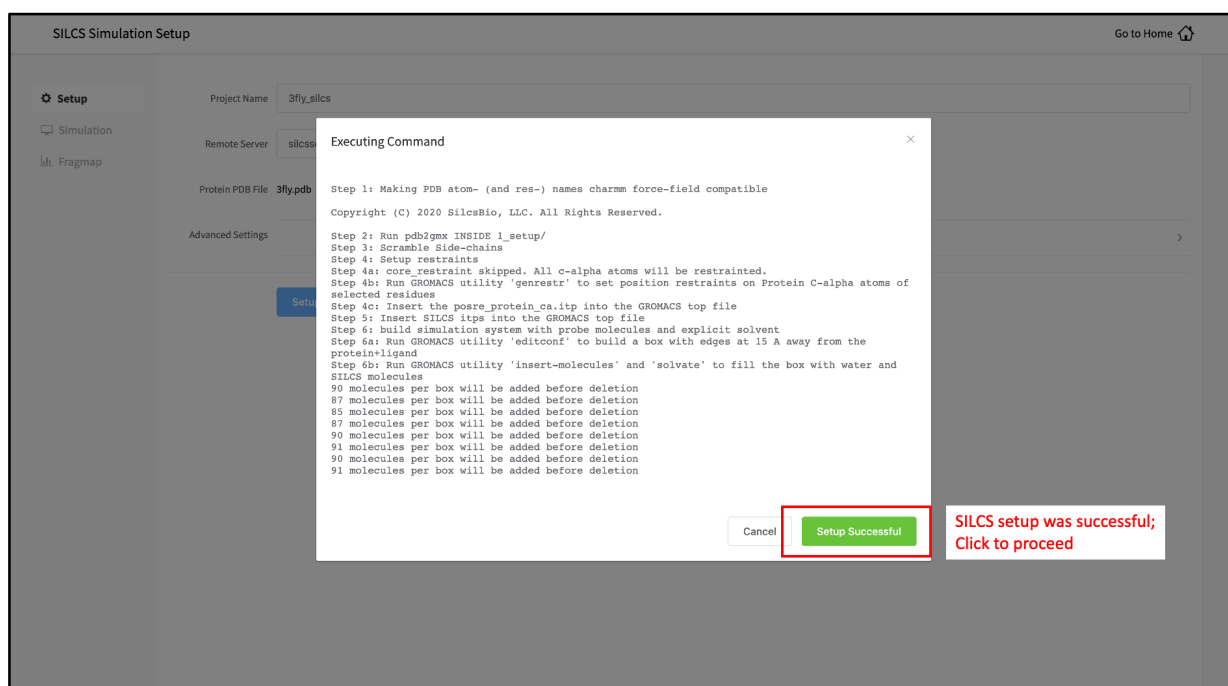
- Project Name:** A text input field containing '3fly_silcs'.
- Remote Server:** A dropdown menu currently set to 'silcsserver'.
- Protein PDB File:** A section with a blue 'select file' button highlighted by a red rectangle. To the right of this button, red text reads: 'Press to select an input PDB on your computer or on a remote server'.
- Advanced Settings:** A section with a right-pointing arrow, indicating expandable options.
- Buttons:** At the bottom of the form are two buttons: a blue 'Setup' button and a white 'Reset' button.

Next, select a protein PDB file. As described in [File and directory selection](#), choose a file from your local computer (“localhost”) or from any server you had configured through the [Remote server setup](#) process. We recommend cleaning your input PDB file before use, including keeping only those protein chains that are necessary for the simulation, removing all unnecessary ligands, renaming non-standard residues, filling in missing atomic positions, and, if desired, modeling in missing loops.

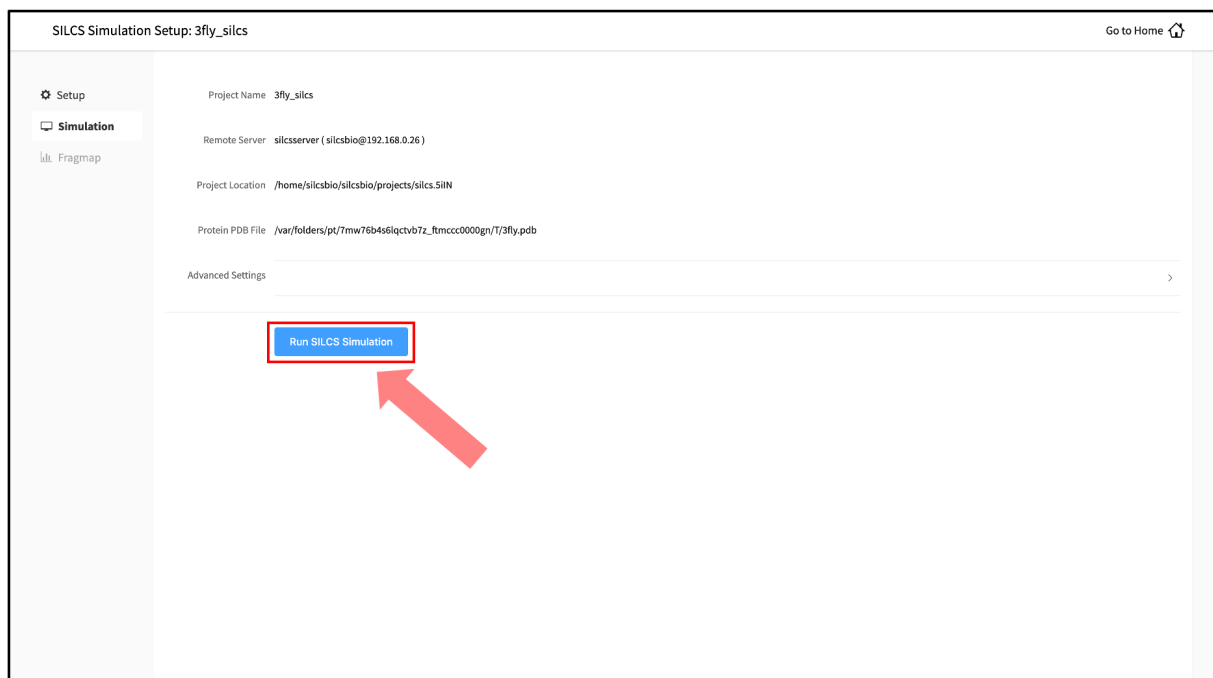
If the GUI detects missing non-hydrogen atoms, non-standard residue names, or non-contiguous residue numbering, it will inform the user and provide a button labeled “Fix?”. If this button is clicked, a new PDB file with these problems fixed and with `_fixed` added to the base name will be created and used. Press the “Setup” button at the bottom of the page. The GUI will contact the remote server and perform the SILCS GCMC/MD setup process.



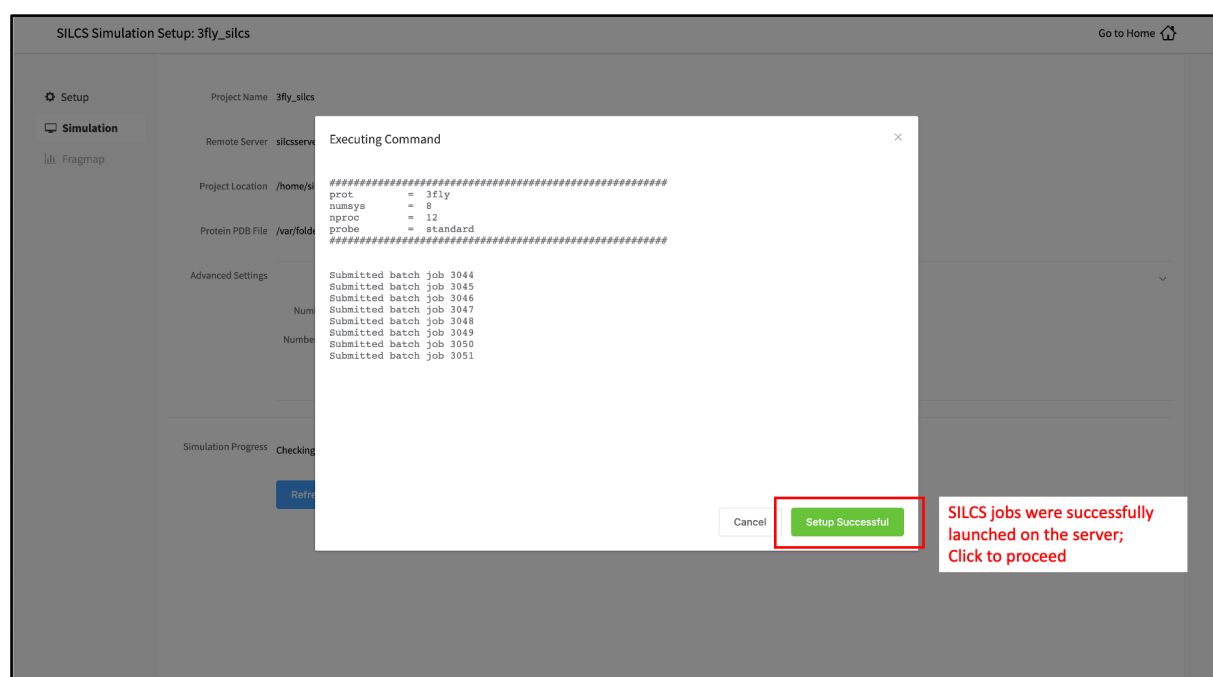
- During setup, the program automatically performs several steps: building the topology of the simulation system, creating metal-protein bonds if metal ions are found, rotating side chain orientations to enhance sampling, and putting probe molecules around the protein. To complete the entire process may take up to 10 minutes depending on the system size. A green “Setup Successful” button will appear once the process has successfully completed. Press this button to go to the next step.



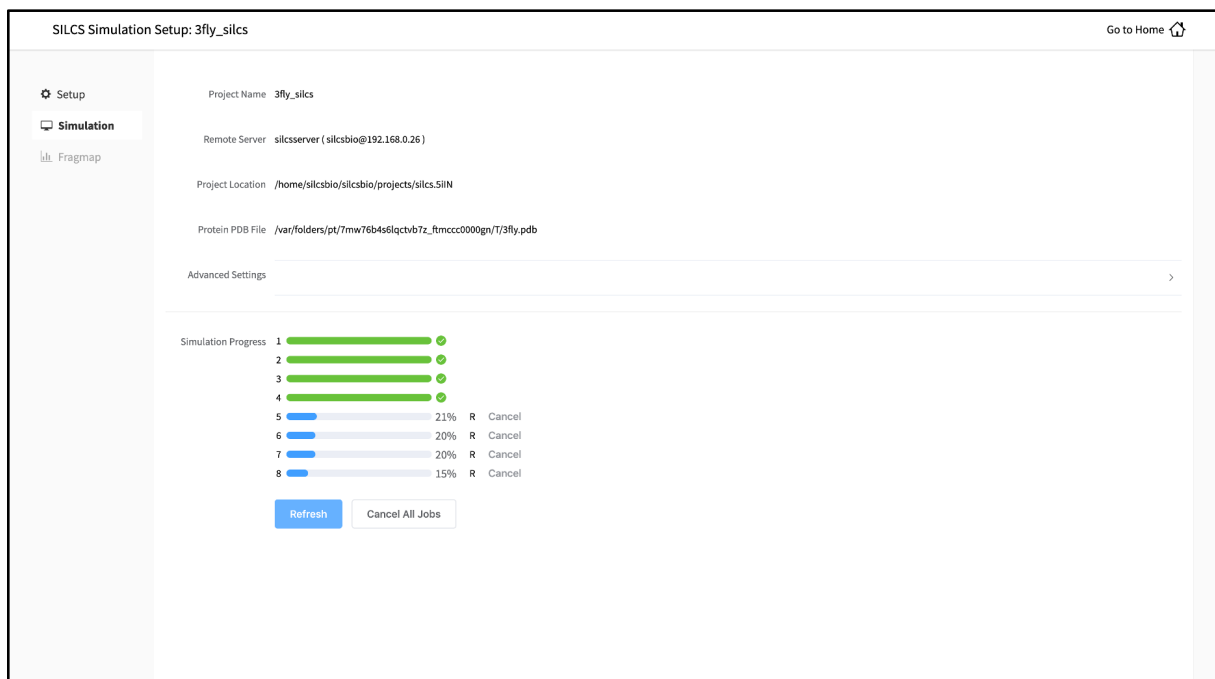
4. Your SILCS GCMC/MD simulation can now be started by clicking the “Run SILCS Simulation” button. Before running, you may wish to double check that you have chosen the desired file folder on the remote server and that it has 100+ GB of storage space.



Compute jobs will be submitted to the queueing system on the server.



The status of each job is shown next to its progress bar: “Q” for queued and “R” for running. When a job successfully completes, its progress bar will turn green.

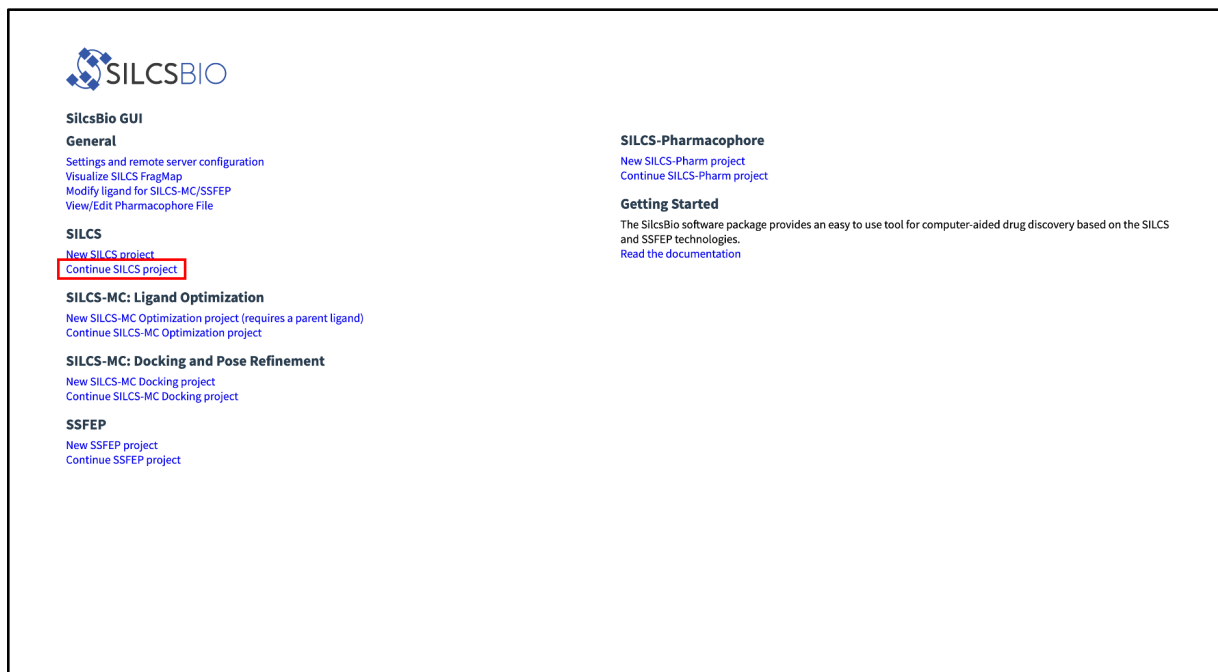


Once your jobs are in progress, in either the queued or running state, you may safely quit the SilcsBio GUI or go back to the Home page to do other tasks.

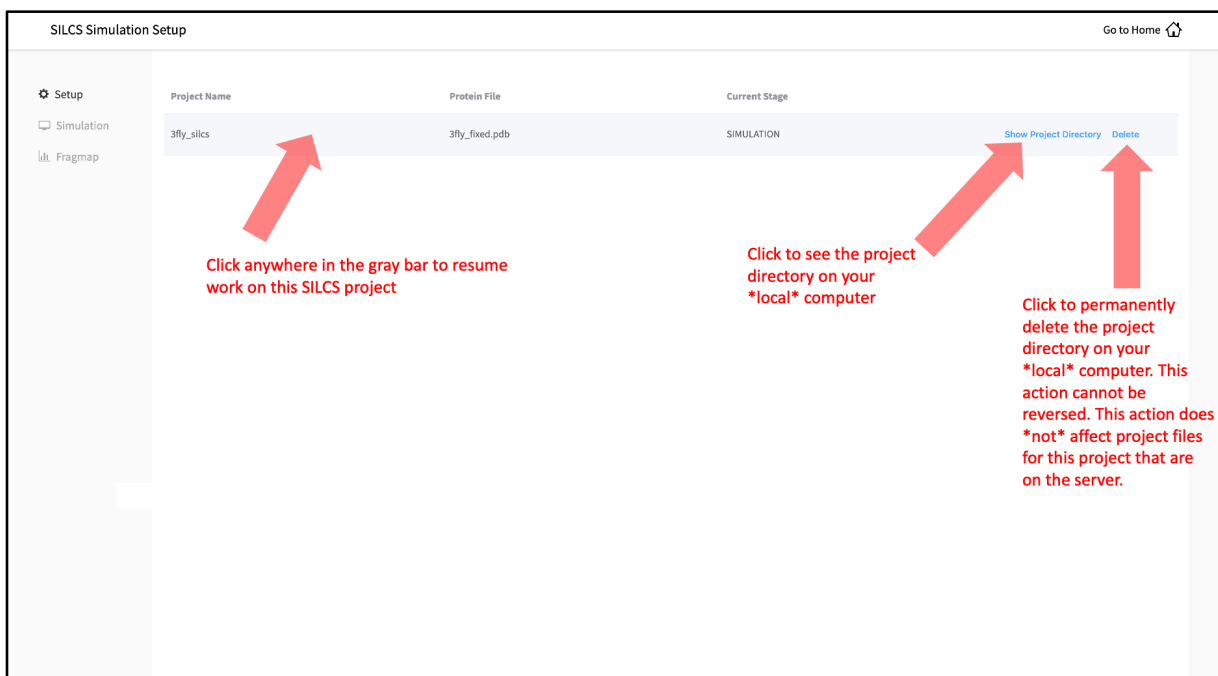
If a job encounters an error or you cancel it before it finishes, its progress bar will turn red. “Restart” will appear next to the progress bar. Pressing “Restart” will resubmit the job to the queue and continue from the last cycle of the calculation so that previous progress on that job is not lost.

Tip: The progress bar of a restarted job will reset to “0%” until the first GCMC/MD cycle successfully completes. At that point the progress bar will update to reflect all progress prior to the restart. Depending on the size of your simulation system and the speed of your hardware, this may take from a few minutes to an hour or more.

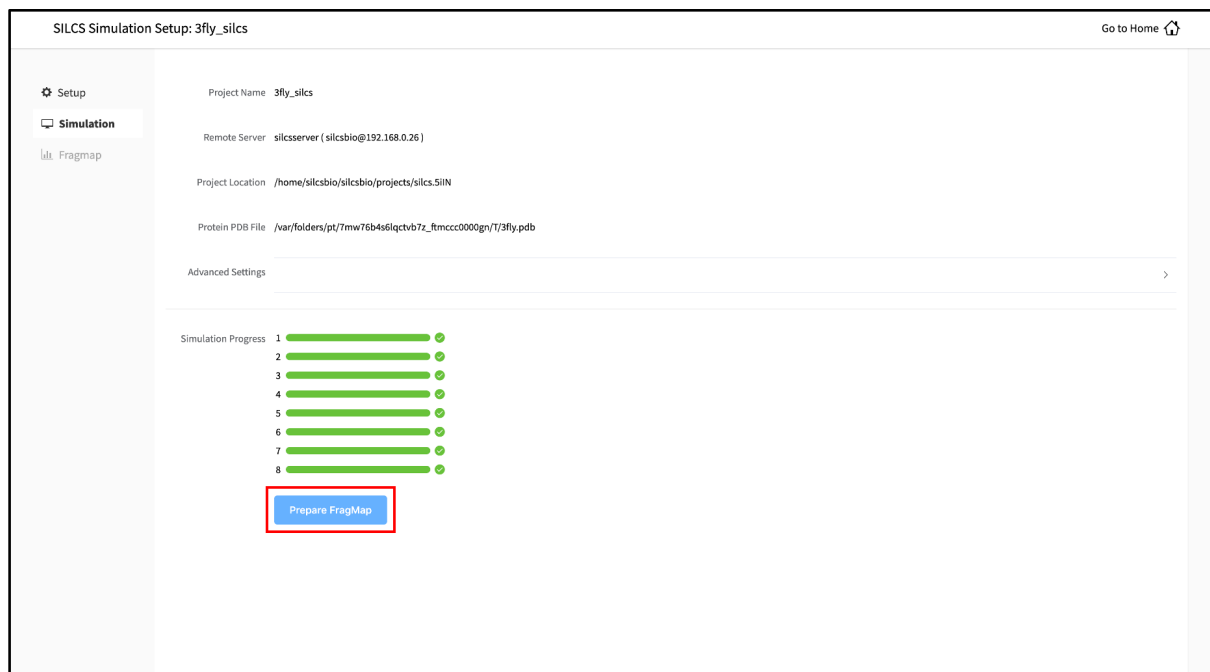
- To see a full listing of all of your projects, select *Continue SILCS project* from the Home page. This will show the complete list of all SILCS projects you have set up on the local machine where you are currently running the SilcsBio GUI, as well as the status of each project.



To resume work on a project or check the status of associated compute jobs you previously started, simply click the project name in the list.



- Once your SILCS project compute jobs are finished, the GUI can be used to create FragMaps and visualize them. Green progress bars indicate successful job completion. Once all progress bars are green, the “Prepare FragMap” button will appear.

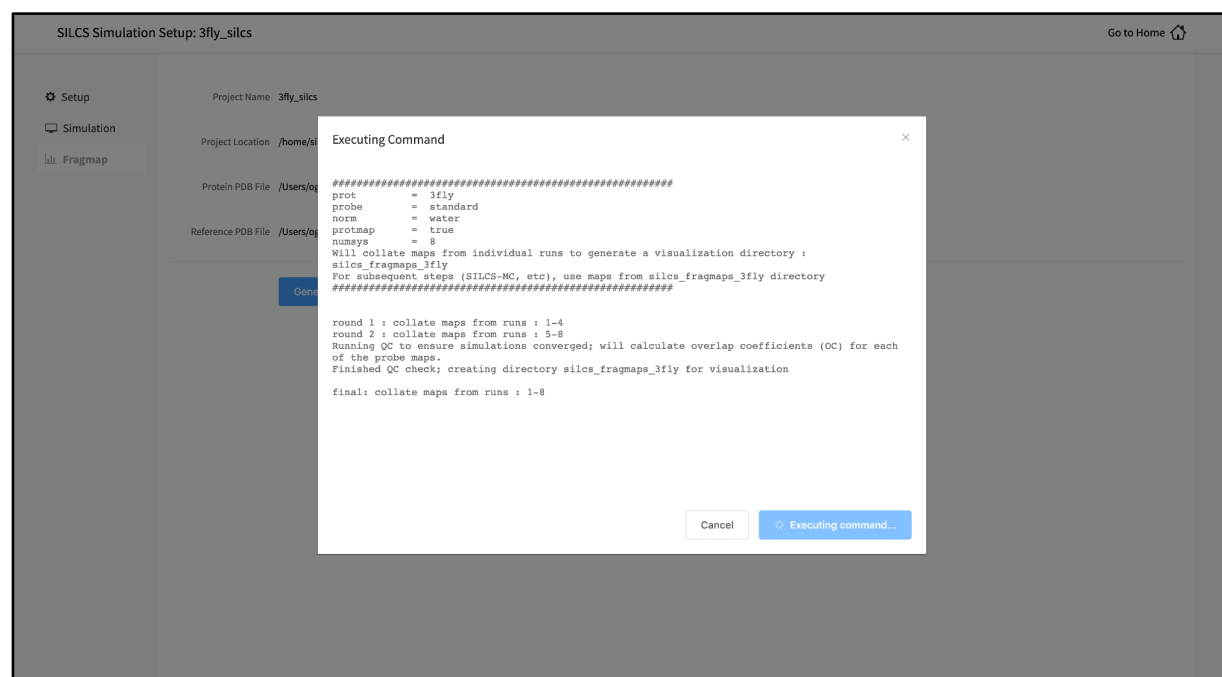


Press this button, and on the next screen, confirm your “Reference PDB File.” FragMaps will be created in the coordinate reference frame of this file. Generally, this reference PDB file is the same as the protein PDB file. A different file with the protein in a different orientation can be selected if you want to generate FragMaps relative to that orientation. Click “Generate FragMap” to generate FragMaps and download them to the local computer for visualization.

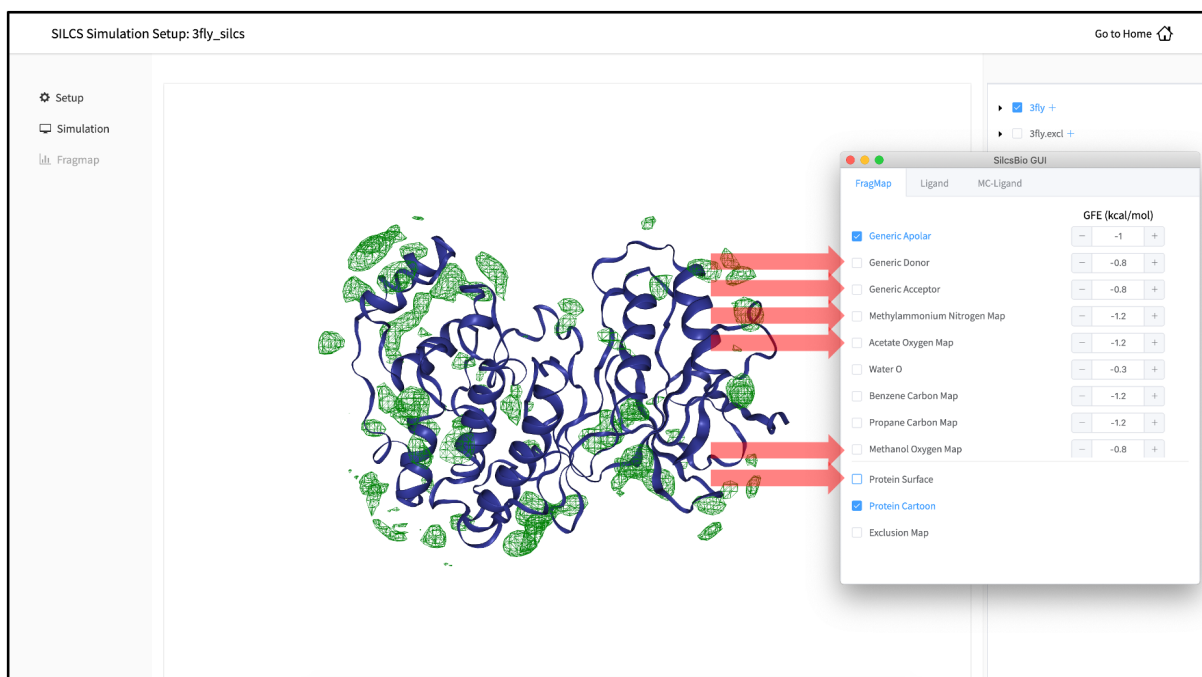
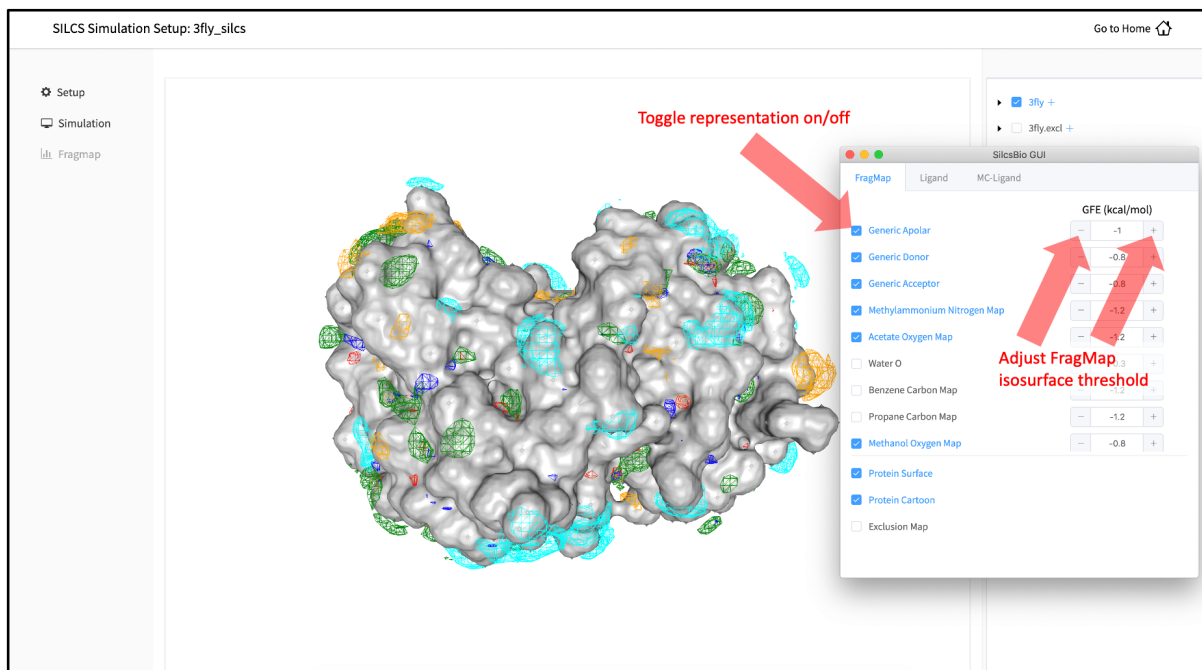
Tip: If you plan to compare FragMaps from two different protein structures, you will want to generate them with the same orientation. In that case, pre-align your input structures with each other and use these aligned coordinates as your Reference PDB Files.

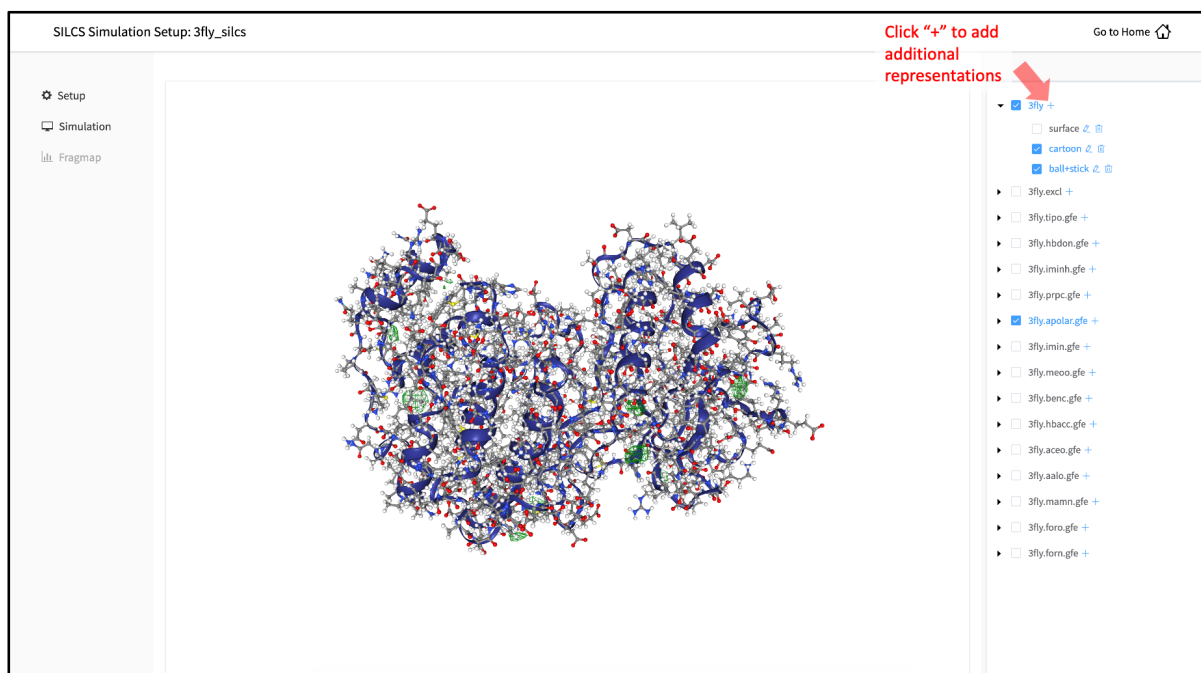
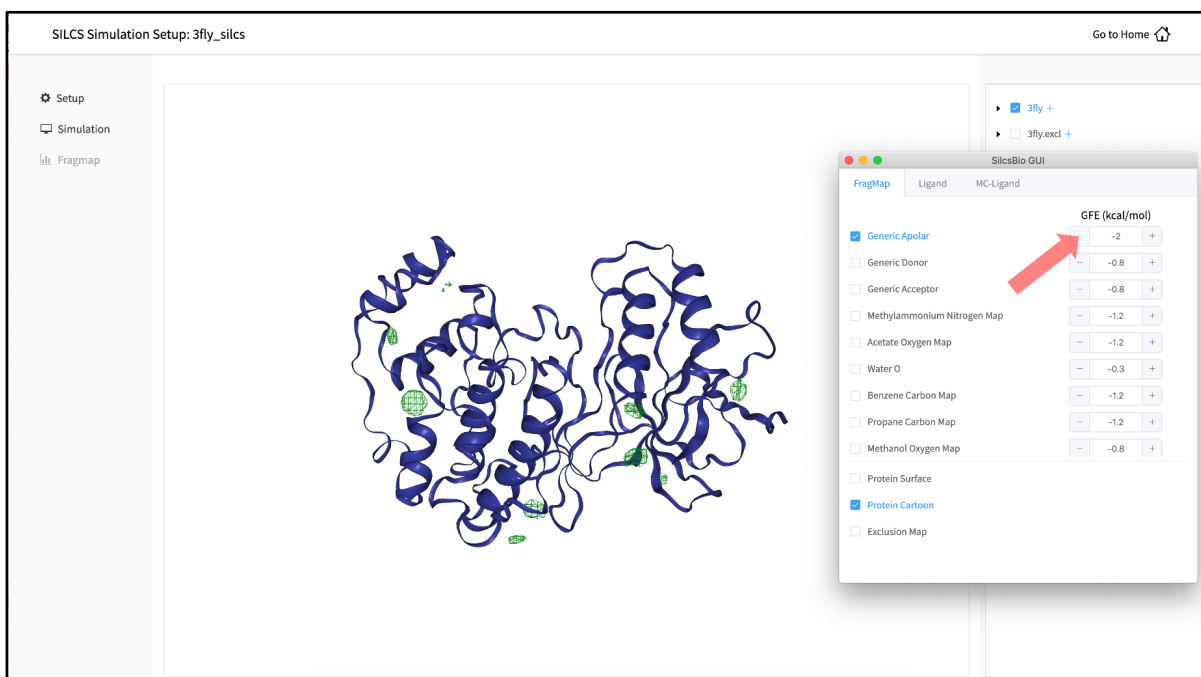


Processing the GCMC-MD trajectories will take 10-20 minutes, with the GUI providing updates on progress from the server during the process.



Once completed, the GUI will automatically copy the files from the server to the local computer and load them for visualization.





SILCS FragMaps are the basis for a host of functionality. The FragMaps can be used for optimization of a parent ligand (*SILCS-MC: Ligand Optimization*), docking of ligands and refinement of existing docked poses (*SILCS-MC: Docking and Pose Refinement*), creation of pharmacophore

models (*SILCS-Pharm: Receptor-Based Pharmacophore Models from FragMaps*), and detection of hotspots and fragment-based drug design (*SILCS-Hotspots: Fragment Binding Sites Including Allosteric Sites*).

For additional details on SILCS, please see *SILCS: Site Identification by Ligand Competitive Saturation*.

3.4 SSFEP simulation from the GUI

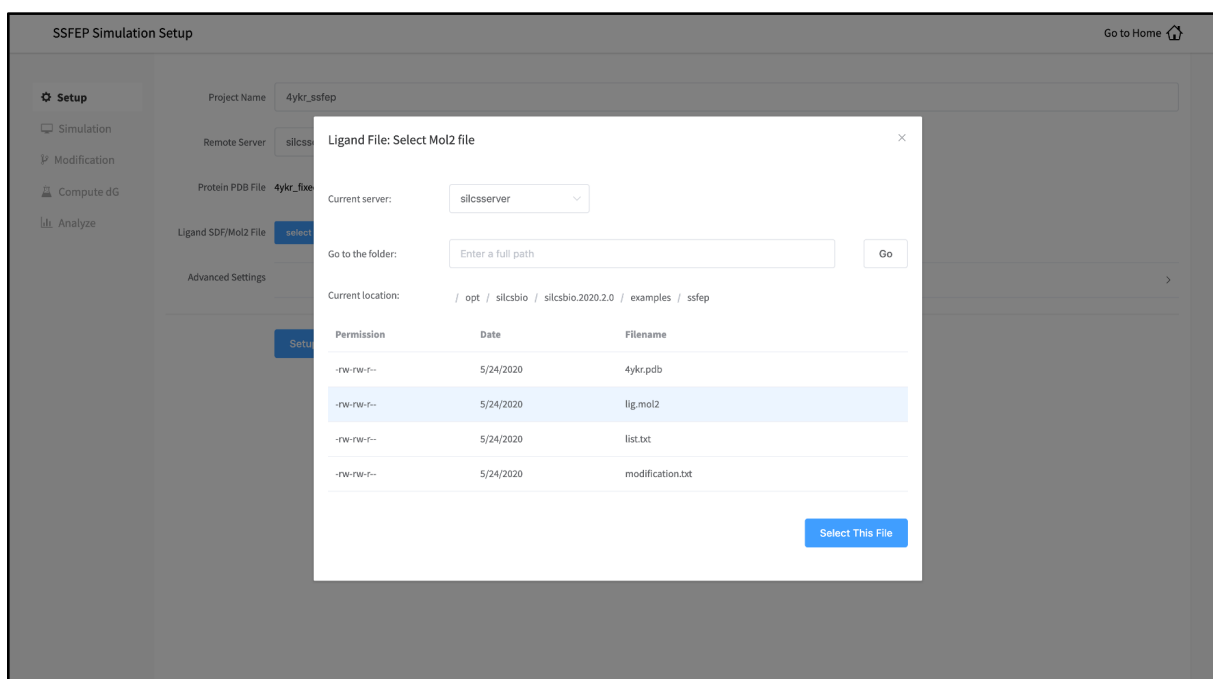
To begin a new SSFEP project, follow these steps:

1. Select *New SSFEP project* from the Home page.
2. Enter a project name, and select the remote server where compute jobs will run. Typical SSFEP simulations produce output files in excess of 20 GB, so please select a project location file folder with appropriate storage capacity.

Next, select a protein PDB file and a ligand file as described in *File and directory selection*. The ligand should be aligned to the binding pocket in the accompanying protein PDB file. We recommend cleaning your PDB file before use, including keeping only those protein chains that are necessary for the simulation, removing all unnecessary ligands, renaming non-standard residues, filling in missing atomic positions, and, if desired, modeling in missing loops.

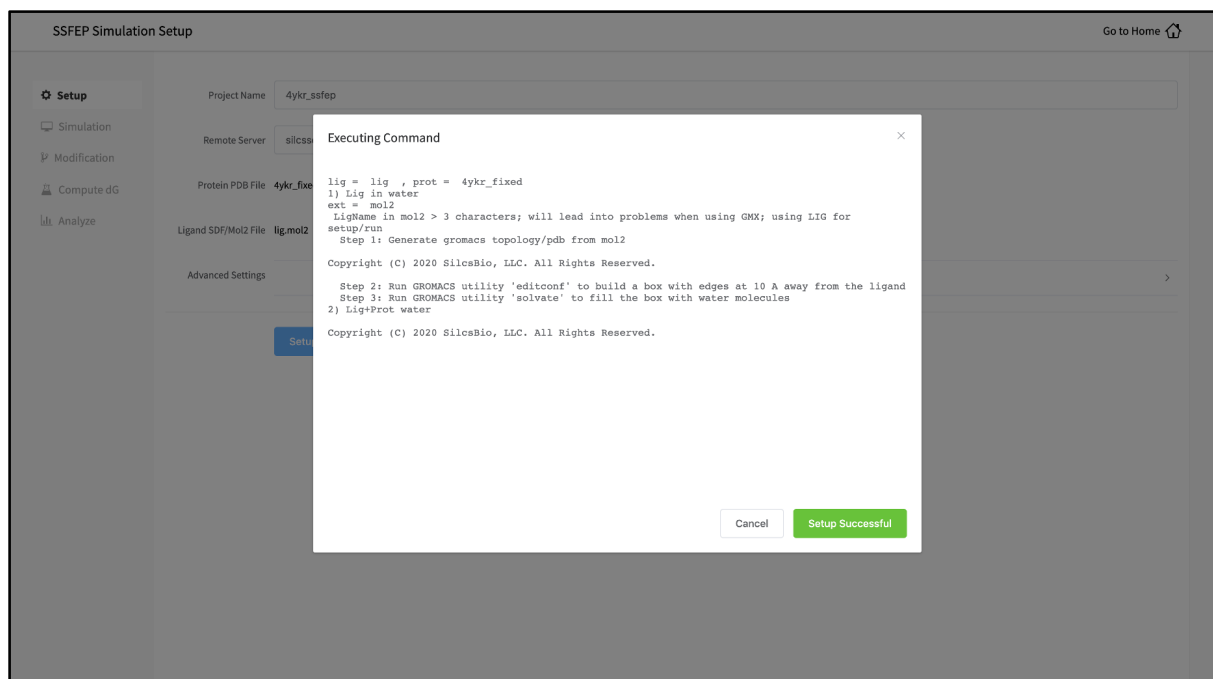
If the GUI detects missing non-hydrogen atoms, non-standard residue names, or non-contiguous residue numbering, it will inform the user and provide a button labeled “Fix?”.

If this button is clicked, a new PDB file with these problems fixed and with `_fixed` added to the base name will be created and used in the SSFEP simulation.

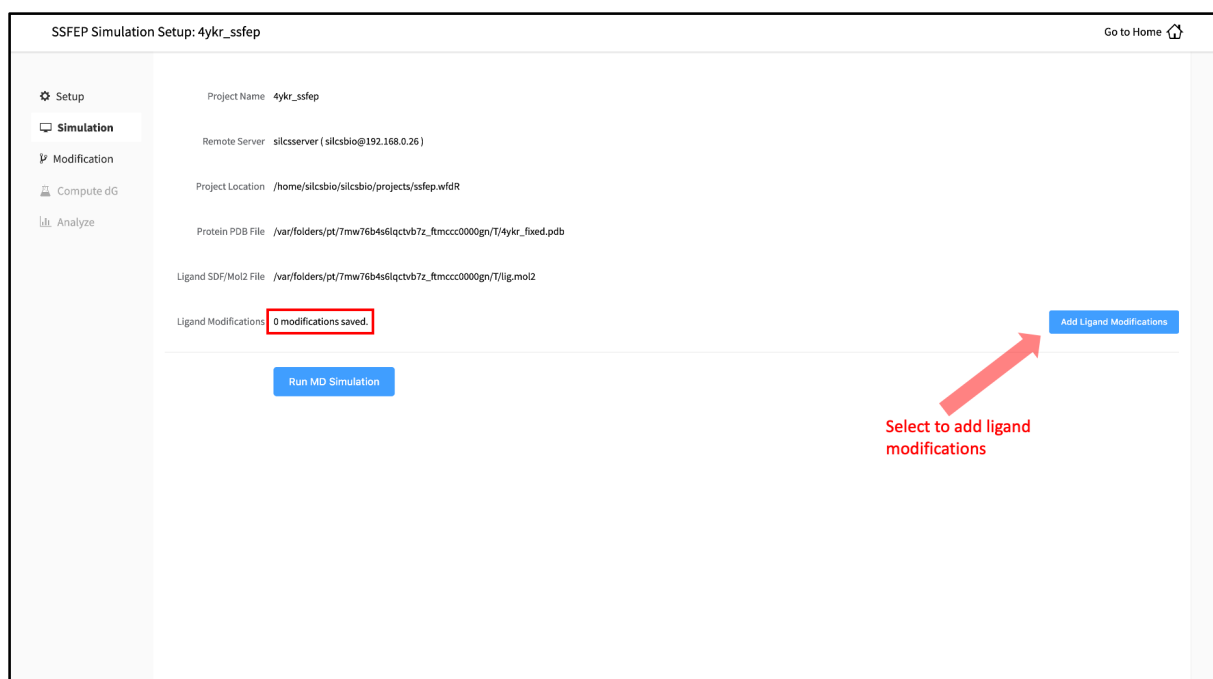


3. Once all information is entered correctly, press the “Setup” button at the bottom of the page. The GUI will contact the remote server and perform the SSFEP setup process.

During setup, the program automatically performs several steps including building the topology of the simulation system and creating metal-protein bonds if metal ions are found. To complete the entire process may take up to 10 minutes depending on the system size. A green “Setup Successful” button will appear once the process has successfully completed. Press this button to proceed.

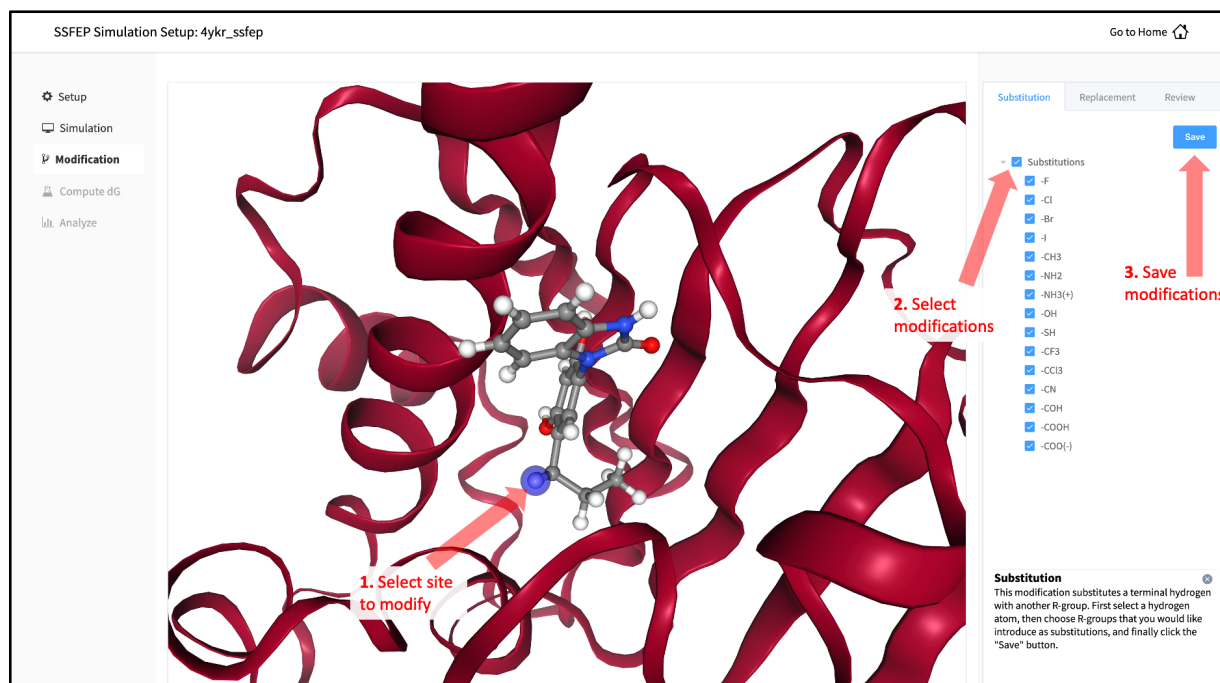


4. You can now prepare your ligand modifications with the “Add Ligand Modifications” button.



There are two major modification types, Substitution and Replacement, available in the GUI. Substitution is used to substitute a hydrogen with another functional group. Replacement is used to replace an atom in a ring with another functional group that preserves the ring.

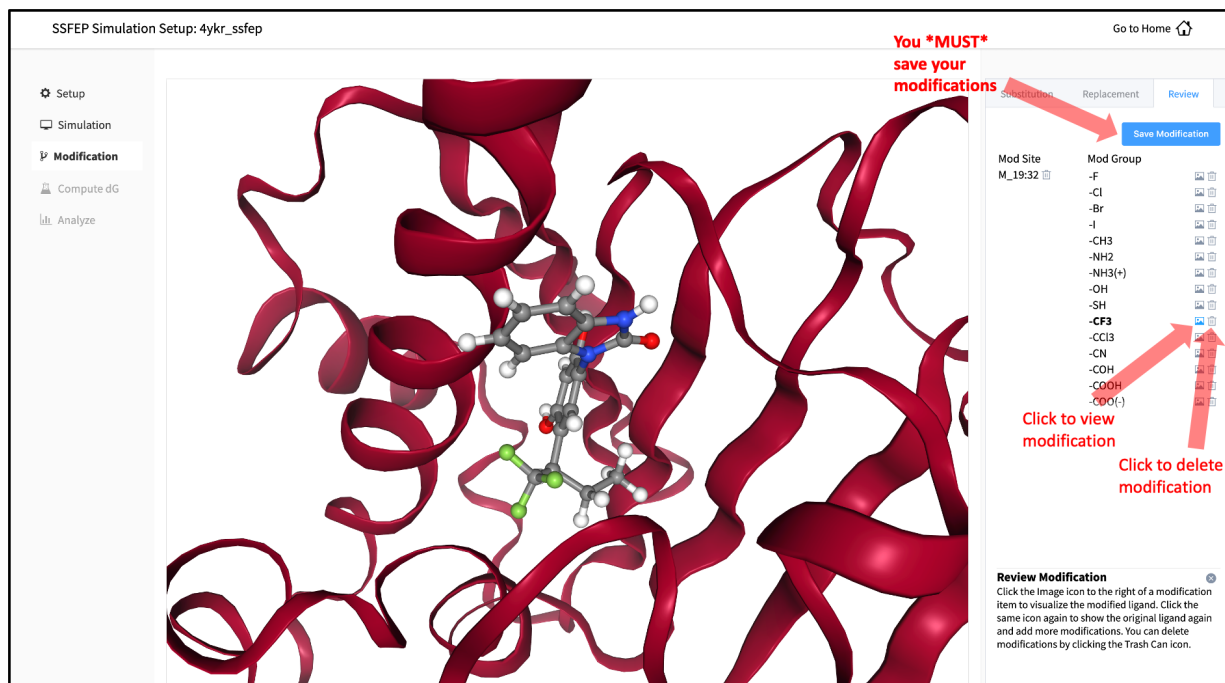
In the visualization window, select the atom to be modified. Then, select your desired modifications from the “Substitution” or the “Replacement” tab in the right-hand panel. Pressing the “Save” button in the panel will update your list of modifications.



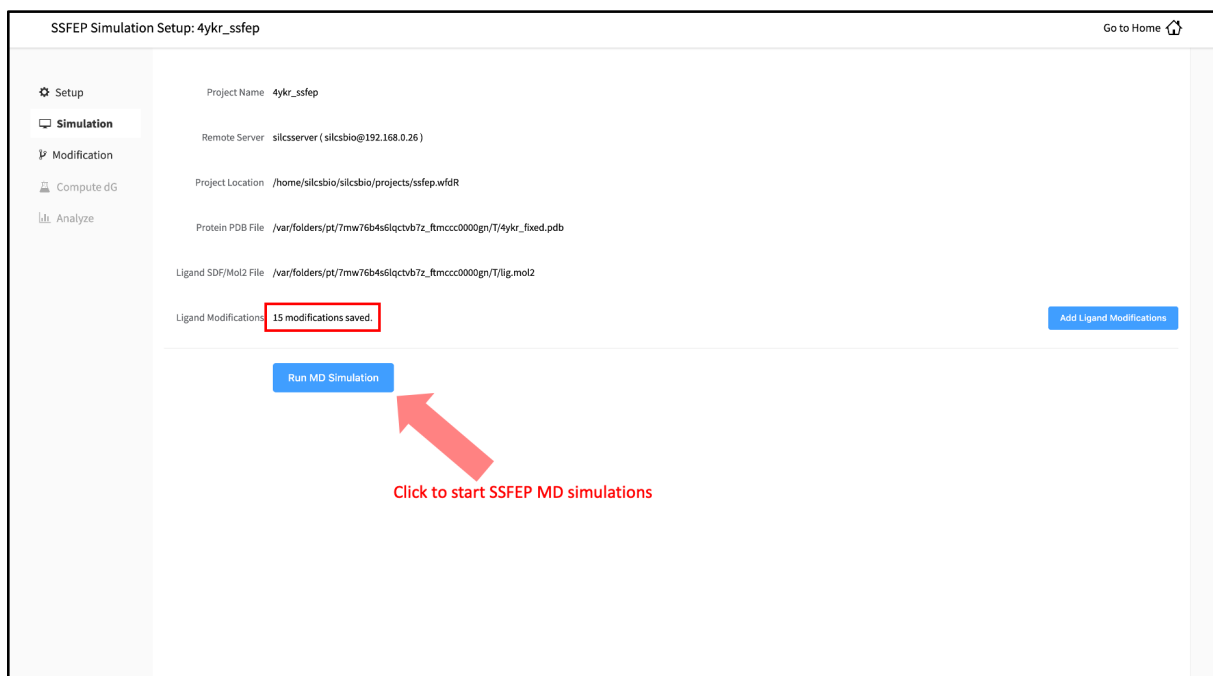
The list of modification types in the GUI covers a broad range of chemical functionality. Custom modifications can be made using the Command Line Interface (CLI) as detailed in *SSFEP: Single Step Free Energy Perturbation*.

6. Use the “Review” tab to confirm your desired modifications.

Valid modifications will have a small Image icon as well as a small Trash Can icon. Clicking on the Image icon will show the modification in the center panel. Clicking on it again will show the parent ligand. Clicking on the Trash Can icon will delete the proposed modification from your list. You can go back to the “Substitution” and “Replacement” tabs to add to your list. Once you have completed your list of modifications, **you must press the “Save Modification” button in the “Review” tab to actually save the list of modifications for your project.**



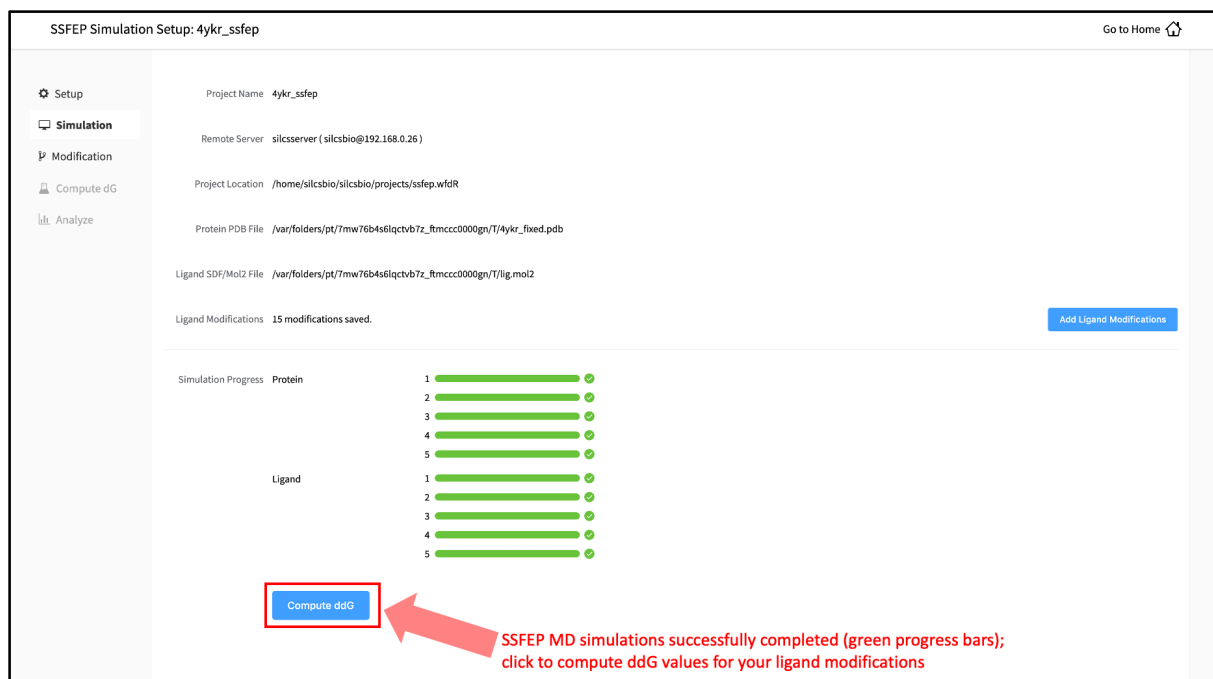
4. Your SSFEP simulation can now be started by selecting “Simulation” from the left-hand pane and then clicking the “Run MD Simulation” button in the resulting screen. Before running, you may wish to double check that you have chosen the desired file folder on the remote server and that it has 20+ GB of storage space. There are two parts to SSFEP: a compute-intensive MD simulation and a very rapid $\Delta\Delta G$ calculation. The compute-intensive MD may take several hours, and is done only once. The rapid $\Delta\Delta G$ calculation part relies on the MD results and is able to test thousands of functional group modifications to your parent ligand in under an hour. Should you wish to test additional modifications to your parent ligand at a later time in the project, there is no need to re-run the compute-intensive MD, which makes SSFEP a very efficient method.



10 compute jobs will be submitted to the queueing system (five for the ligand and five for the protein:ligand complex) on your remote server. Job progress will be displayed in this same window. The status of each job is shown next to its progress bar: “Q” for queued and “R” for running. At this point, your jobs are in progress and you may safely quit the SilcsBio GUI or go back to the Home page to do other tasks.

If a job encounters an error and does not finish, a “restart” button will appear next to the status. If the restart button is used, the job will be resubmitted to the queue and continue from the last cycle of the calculation.

8. Once the MD simulation stage has finished, use the GUI to automatically analyze your list of modifications and create a chart.



SSFEP Simulation Setup: 4ykr_ssfep

Go to Home

Setup

Simulation

Modification

Compute dG

Analyze

Project Name: 4ykr_ssfep

Remote Server: silcsserver (silcsbio@192.168.0.26)

Project Location: /home/silcsbio/silcsbio/projects/ssfep.wfdr

Protein PDB File: /var/folders/pt/7mw76b4s6lqctvb7z_fmccc0000gn/T/4ykr_fixed.pdb

Ligand SDF/Mol2 File: /var/folders/pt/7mw76b4s6lqctvb7z_fmccc0000gn/T/lig.mol2

Ligand Modifications: 15 modifications saved.

Add Ligand Modifications

Simulation Progress

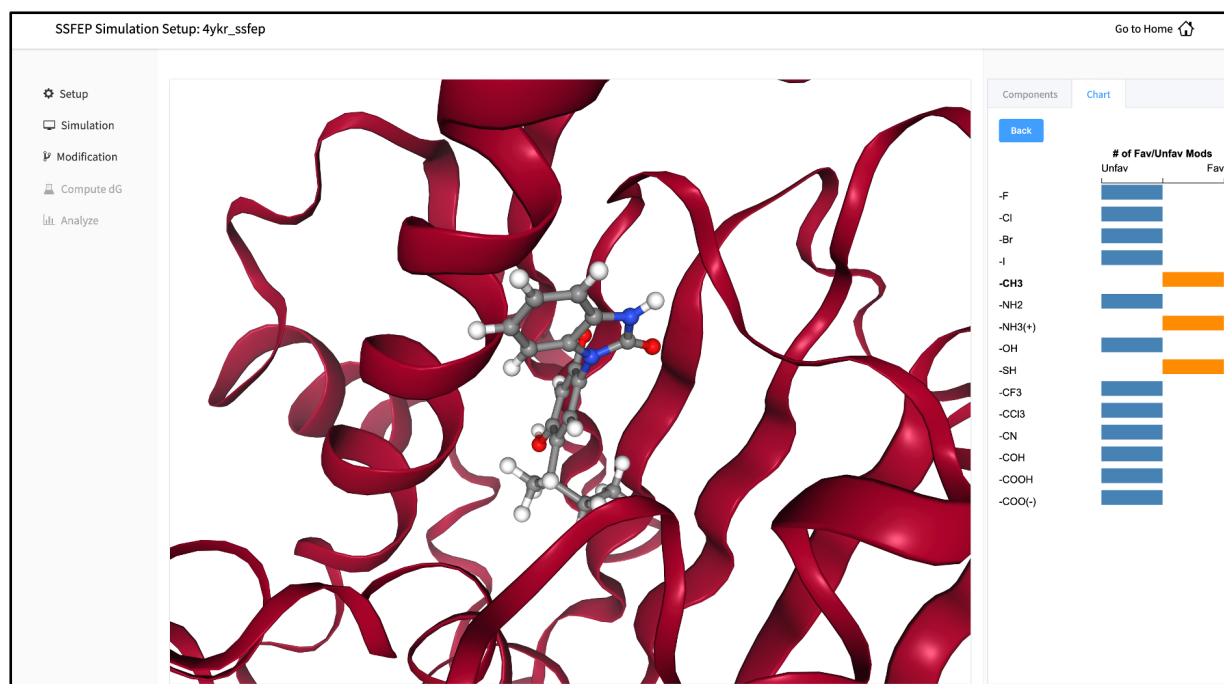
Protein

Ligand

Compute ddG

SSFEP MD simulations successfully completed (green progress bars); click to compute ddG values for your ligand modifications

SSFEP is designed to evaluate small modifications and results are best interpreted qualitatively. Therefore GUI-created charts indicate the predicted change in direction of the binding affinity relative to the parent ligand.



SSFEP Simulation Setup: 4ykr_ssfep

Go to Home

Setup

Simulation

Modification

Compute dG

Analyze

Components

Chart

Back

of Fav/Unfav Mods

Unfav Fav

-F

-Cl

-Br

-I

-CH3

-NH2

-NH3(+)

-OH

-SH

-CF3

-Cl3

-CN

-COH

-COOH

-COO(-)

For additional details on SSFEP, please see *SSFEP: Single Step Free Energy Perturbation*.

COMMAND LINE INTERFACE QUICKSTART

This chapter provides a step-by-step introduction on how to use the SilcsBio Command Line Interface (CLI). Example commands assume a Bash shell is being used.

4.1 SILCS simulation from the command line

1. Set the environment variables required to access the software:

```
export GMXDIR=<gromacs/bin>
export SILCSBIODIR=<silcsbio>
```

2. Set up the SILCS simulations:

```
${SILCSBIODIR}/silcs/1_setup_silcs_boxes prot=<Protein PDB>
```

To determine if the setup is complete check that the 10 PDB files required for the simulations are available using the command `ls 1_setup/*_silcs.*.pdb`

3. Submit the SILCS GCMC/MD jobs to the queue:

```
${SILCSBIODIR}/silcs/2a_run_gcmd prot=<Protein PDB>
```

This will submit 10 jobs to the queue. To check job progress, use:

```
${SILCSBIODIR}/silcs/check_progress
```

This will provide a summary list of the SILCS jobs consisting of the full job path, the job number, the task ID, the job status, and the current/total number of GCMC/MD cycles. Job status values are: Q queued, R running, E successfully completed, F failed, NA not submitted.

4. When the GCMC/MD jobs are finished, generate FragMaps:

```
${SILCSBIODIR}/silcs/2b_gen_maps prot=<Protein PDB>
```

This command will submit 10 jobs to the queue for calculating the occupancy maps from individual runs. Once they are done, use the following command to create the FragMaps:

```
${SILCSBIODIR}/silcs/2c_fragmap prot=<Protein PDB>
```

This command will create a `silcs_fragmap_<Protein PDB>` folder, which contains final FragMap and VMD/PyMol scripts for easy visualization:

```
pymol view_maps.pml # PyMol
vmd -e view_maps.vmd # VMD
```

Additional files that are created include those needed for subsequent SILCS-MC docking and the file `overlap_coefficient.dat`. The overlap coefficients in this file are a measure of convergence for SILCS simulations. If the coefficients are less than 0.7, you may consider extending the SILCS simulations.

For visualization in MOE, `2c_fragmaps` needs to be re-run with extra option.

```
${SILCSBIODIR}/silcs/2c_fragmap prot=<Protein PDB> cns=true
```

This command will create a CNS format map files and `view_maps.svl` script file. To visualize the maps in MOE, start MOE and open “Surfaces and Maps” panel, and type the following command in the SVL console.

```
run 'view_maps.svl'
```

For additional details, please see *SILCS: Site Identification by Ligand Competitive Saturation*.

4.2 SSFEP simulation from the command line

1. Set the environment variables required to access the software:

```
export GMXDIR=<gromacs/bin>
export SILCSBIODIR=<silcsbio>
```

2. Set up the SSFEP simulations:

```
${SILCSBIODIR}/ssfep/1_setup_ssfep lig=<Ligand Mol2/SDF file> prot=
→<Protein PDB>
```

To determine if the setup is completed check that the PDB files required for the simulations are available using the command `ls 1_setup/*/*_gmw_wat.pdb`. The listing should show PDB files for the ligand alone and for the protein/ligand complex.

3. Submit the SSFEP MD simulation jobs to the queueing system:

```
{SILCSBIODIR}/ssfep/2_run_md_ssfep lig=<Ligand Mol2/SDF file>
→prot=<Protein PDB>
```

This will submit 10 jobs to the queue, 5 for the protein:ligand complex and 5 for the ligand. To check job progress, use:

```
{SILCSBIODIR}/ssfep/check_progress
```

This will provide a summary list of the SSFEP jobs consisting of the full job path, the job number, the task ID, the job status, and the current/total number of SSFEP cycles. Job status values are: Q queued, R running, E successfully completed, F failed, NA not submitted.

4. When the SSFEP MD simulation jobs are finished, use the ligand modification files to submit the $\Delta\Delta G$ calculations:

```
{SILCSBIODIR}/ssfep/3a_setup_modifications lig=<Ligand Mol2/SDF
→file> prot=<Protein PDB> mod=<modification file>
```

This command will submit 10 jobs, each of which processes one of the MD trajectories for all modifications in the modification file. Depending on the number and sizes of the modifications, this step may take minutes to several hours to complete.

Once completed, use the following command to collate the results for all of the modifications:

```
{SILCSBIODIR}/ssfep/3b_calc_ddG_ssfep mod=<modification file>
```

This command will create a `lig_decor.csv` file, which contains the free energy change for each modification relative to the parent ligand. SSFEP is designed to evaluate small modifications and results are best interpreted qualitatively. Therefore it is recommended that only the sign of the change, and not the magnitude, be used to inform decision making: values < 0 indicate a modification predicted to be favorable.

For additional details, please see *SSFEP: Single Step Free Energy Perturbation*.

SILCSBIO SOFTWARE INSTALLATION

5.1 Minimum hardware requirement

SilcsBio software requires relatively robust computational resources for the molecular dynamics (MD) components of the SILCS and SSFEP protocols. For example, computing SILCS FragMaps for a 35 kDa target protein takes 80-90 hours of walltime when run in parallel on ten compute nodes, each equipped with 8 3-GHz CPU cores. The software can take advantage of GPU acceleration: the addition of a single NVIDIA GeForce RTX 2070 GPU to each node will reduce the walltime to 24-48 hours. In the case of SSFEP, walltime using these GPU-equipped nodes will be 3-4 hours.

SilcsBio software is designed to run the compute-intensive MD on a cluster using a cluster queue management system such as OpenPBS, Sun Grid Engine, or SLURM. With both SILCS and SSFEP, subsequent evaluation of relative ligand affinities takes seconds to minutes on a single CPU core, allowing for modifications to be rapidly evaluated for a large number of ligands to a given target.

For customers without ready access to an appropriate in-house computing cluster, SilcsBio offers three possible solutions. The first solution is the SilcsBio Workstation. The SilcsBio Workstation combines the SilcsBio server and GUI software with high-performance GPU-computing hardware in a quiet, sleek form factor for use in an office setting. The SilcsBio Workstation is a complete turn-key solution that is ready to plug in to a standard wall electrical socket. The second solution is for SilcsBio to perform computations as a service and supply data to the customer for subsequent in-house analysis. For this service, in the case of SILCS, SilcsBio requires only the structure of the target, and, in the case of SSFEP, only the structure of the protein-parent ligand complex. Depending on the choice of SSFEP or SILCS, no intellectual property disclosure to SilcsBio in the form of proposed chemical modifications to the parent ligand (SSFEP) or even the parent ligand itself (SILCS) is required. The third solution is for SilcsBio to assist customers with setting up their own virtual cluster using Amazon Web Services. Please contact info@silcsbio.com for additional information on these solutions.

5.2 Software requirement

SILCS FragMap generation and SSFEP calculations use the MD simulation package GROMACS. Although the SilcsBio software is compatible with GROMACS version 5.1.0 and later, we recommend GROMACS version 2019.6. The package can be obtained at <http://manual.gromacs.org/documentation/2019.6/download.html> and must be installed in order to use the SilcsBio software package.

Below is a recommended sequence of commands for general installation of GROMACS (with GPU acceleration):

```
cd <GROMACS source directory>
mkdir build
cd build
cmake .. -DGMX_BUILD_OWN_FFTW=on \
  -DREGRESSIONTEST_DOWNLOAD=on \
  -DGMX_GPU=on \
  -DBUILD_SHARED_LIBS=off \
  -DGMX_PREFER_STATIC_LIBS=on \
  -DGMX_BUILD_SHARED_EXE=off \
  -DCMAKE_INSTALL_PREFIX=<GROMACS install location>
make
make install
```

If your compute nodes do not have GPUs, use the following command:

```
cd <GROMACS source directory>
mkdir build
cd build
cmake .. -DGMX_BUILD_OWN_FFTW=on \
  -DREGRESSIONTEST_DOWNLOAD=on \
  -DBUILD_SHARED_LIBS=off \
  -DGMX_PREFER_STATIC_LIBS=on \
  -DGMX_BUILD_SHARED_EXE=off \
  -DCMAKE_INSTALL_PREFIX=<GROMACS install location>
make
make install
```

Please refer to <http://manual.gromacs.org/documentation/current/install-guide/index.html> for further detail.

5.3 Installing the SilcsBio server software

The SilcsBio server software package is delivered as a zip-compressed file. Unzip and place the files to an accessible location. The files have the following directory structure

```
silcsbio.$VERSION/  
  data/  
  examples/  
  lib/  
  programs/  
  silcs/  
  silcs-hotspots/  
  silcs-mc/  
  silcs-memb/  
  silcs-pharm/  
  ssfep/  
  ssfep-memb/  
  templates/  
  utils/  
  VERSION
```

The top-level `silcsbio.$VERSION/` folder contains software for running SILCS and SSFEP simulations. The `programs/`, `silcs*/`, and `ssfep*/` folders contain executable code, and the `templates/` folder contains templates for job submission and input scripts. Some template files may need to be edited with information for your queuing system.

If you are a system administrator, place the top-level `silcsbio.$VERSION/` folder where it can be accessed by other users, such as `/opt/silcsbio/`. If you are a single user, you may place the folder in your home directory.

For SilcsBio server software to work, the two shell environment variables `GMXDIR` and `SILCSBIODIR` need to be set. To do so, replace `<gromacs/bin>` and `<silcsbio>` with the complete file paths for the corresponding folders:

```
# bash  
export GMXDIR=<gromacs/bin>  
export SILCSBIODIR=<silcsbio>
```

Currently, the SilcsBio server software is compatible only with the Bash shell environment. You may insert the above environment variable settings in `.bashrc` for convenience.

5.4 Installing the SilcsBio Graphical User Interface

The SilcsBio Graphical User Interface (GUI) enables running SILCS and SSFEP simulations and analyzing results through a GUI instead of the command line. The SilcsBio GUI is available for Windows, macOS, and Linux. Please download and install the software on your local desktop or laptop computer.

In addition to providing standalone features such as FragMap visualization and ligand modification, the SilcsBio GUI can set up, launch, manage, and analyze compute-intensive SILCS and

SSFEP simulations. To enable this functionality requires a simple configuration step to allow the GUI to communicate with your SilcsBio server software.

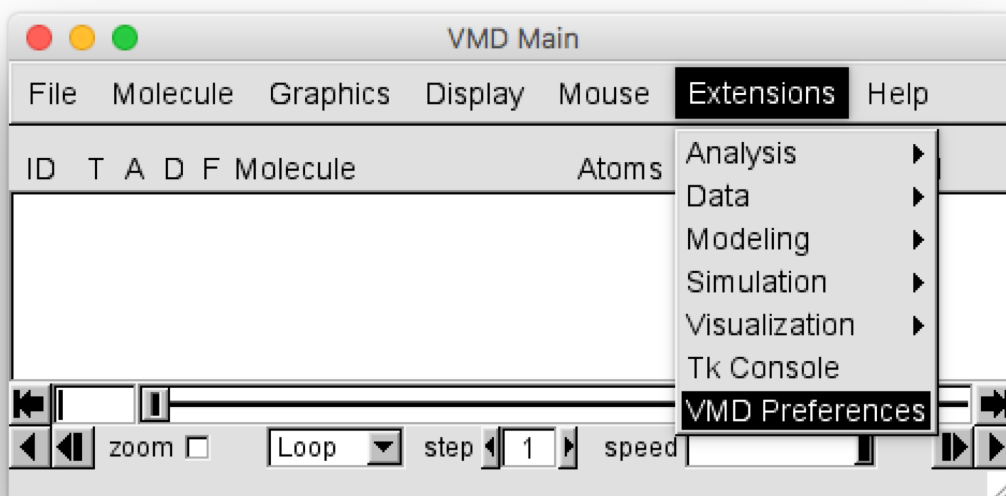
Please follow the *Remote server setup* process as described in *Graphical User Interface Quickstart*. Contact support@silcsbio.com if you need help with this process.

5.5 Installing visualization plugins

Note: These plugins are used for visualization of FragMaps. If you are only interested in SSFEP, or do not intend to use any external programs to visualize FragMaps, you may skip this section.

5.5.1 VMD plugin installation

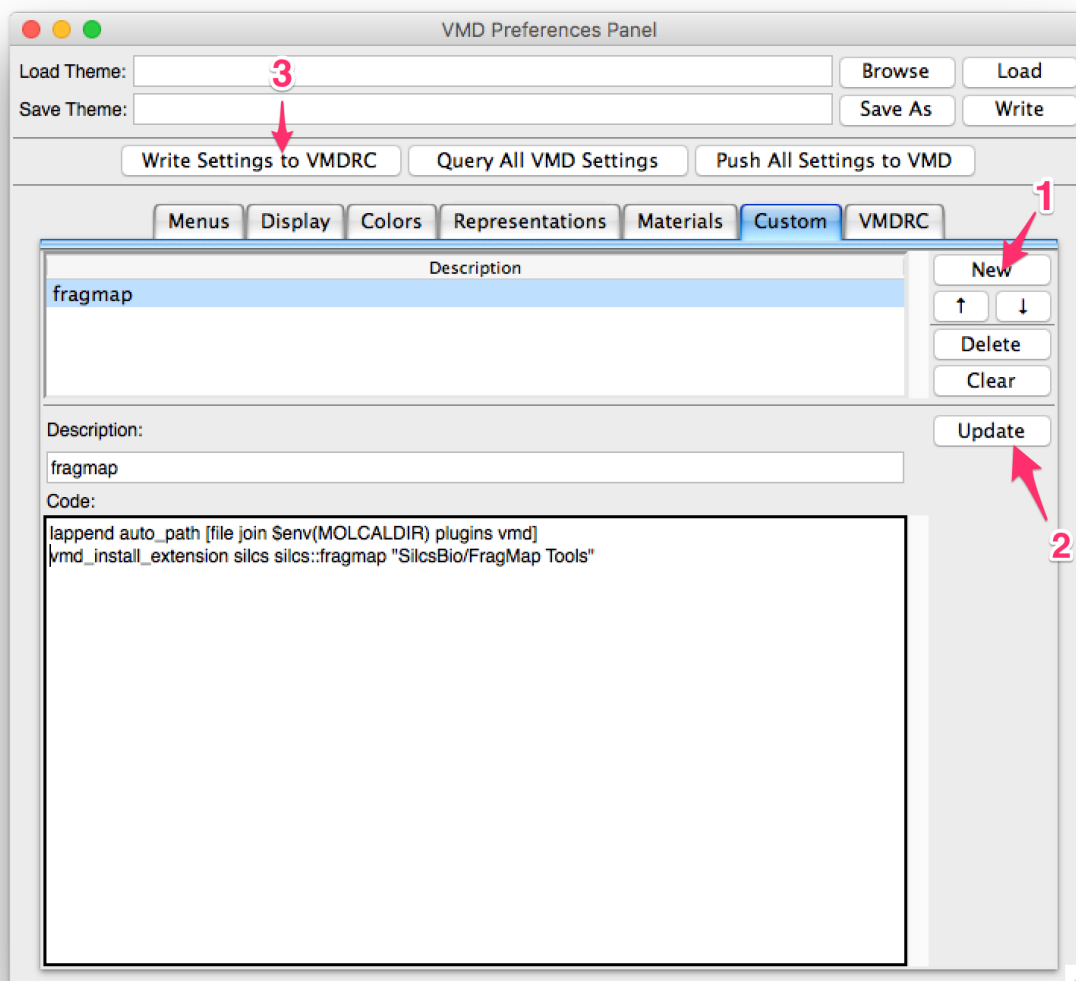
For VMD versions later than 1.9, the plugin can be installed using the VMD Preference menu, which can be found in the “VMD Main” window using the menu selection *Extensions* → *VMD Preferences*:



In the preference window, select the “Custom” tab and press the “New” button. Enter the following lines in the “Code” section:

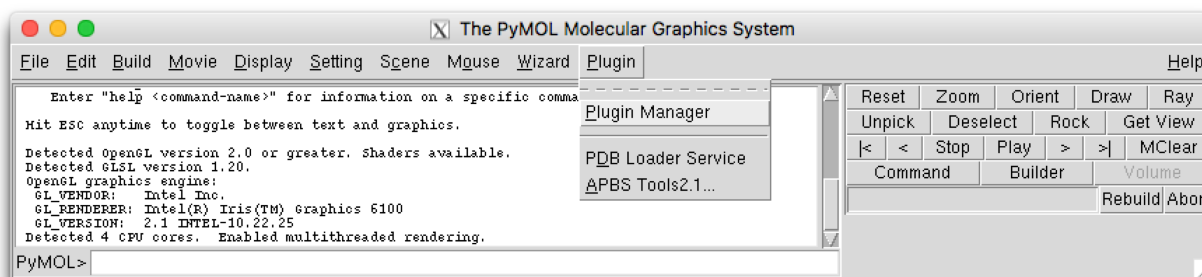
```
lappend auto_path [file join $env(SILCSBIODIR) utils plugins vmd]
vmd_install_extension silcs silcs::fragmap "SilcsBio/FragMap Tools"
```

If you have not set the environment variable `SILCSBIODIR`, replace `$env(SILCSBIODIR)` with the full path to the `silcsbio` folder. Enter an appropriate name for the plugin under “Description:”, then press the “Update” button. Finally, press the “Write Settings to VMDRC” button and then restart VMD to confirm the plugin installation.

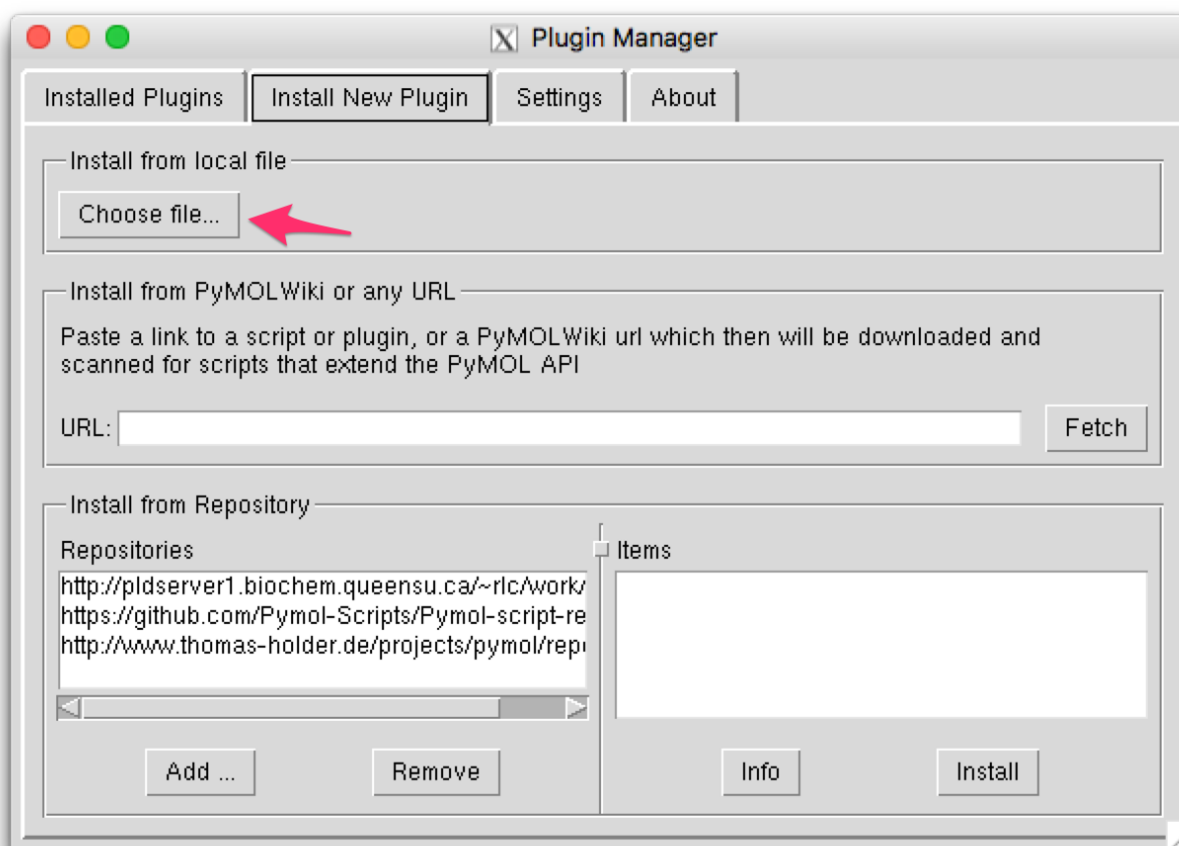


5.5.2 PyMOL plugin installation

The PyMOL plugin can be installed using the plugin manager, which can be found using the PyMol menu selection *Plugin* → *Plugin Manager*:



This will open a plugin manager window. Select the “Install New Plugin” tab. Press the “Choose file...” button in the “Install from local file” section, then choose `silcsbio.2019.1/utis/plugins/pymol/fragmap_tools.py`. This will install the PyMOL plugin. Restart PyMOL to confirm the installation.



FREQUENTLY ASKED QUESTIONS

6.1 1. I installed the software, how do I test if it is correctly installed?

Because different users have different settings and requirements for their clusters or workstations, we provide a general job handling script for you to customize to your needs.

To assist with job submission script customization, example input files are available under the `$SILCSBIODIR/examples` folder.

For SILCS, use the following commands to make sure the software is correctly installed and the job handling script is working. If you are only interested in SSFEP simulations, you may skip to the SSFEP section below.

```
mkdir -p test/silcs
cd test/silcs
cp $SILCSBIODIR/examples/silcs/p38a.pdb .
$SILCSBIODIR/silcs/1_setup_silcs_boxes prot=p38a.pdb
$SILCSBIODIR/silcs/2a_run_gcmd prot=p38a.pdb nproc=1
```

If the script failed to run at the `1_setup-silcs_boxes` step, the software is not correctly installed, whereas if the script failed to run at the `2a_run_gcmd` step, the job handling script needs to be edited. The job handling scripts for SILCS are:

- `templates/silcs/job_mc_md.tmpl`
- `templates/silcs/job_gen_maps.tmpl`
- `templates/silcs/pymol_fragmap.tmpl`
- `templates/silcs/vmd_fragmap.tmpl`
- `templates/silcs/job_cleanup.tmpl`

Typically the header portion of the job submission script requires editing. Please contact support@silcsbio.com if you need assistance.

For SSFEP, use the following commands to make sure the software is correctly installed and the job handling script is working.

```
mkdir -p test/ssfep
cd test/ssfep
cp $SILCSBIODIR/examples/ssfep/* .
$SILCSBIODIR/ssfep/1_setup_ssfep prot=4ykr.pdb lig=lig.mol2
$SILCSBIODIR/silcs/2_run_md_ssfep prot=4ykr.pdb lig=lig.mol2 ↵
↵nproc=1
```

If the script failed to run at the `1_setup_ssfep` step, the software is not correctly installed, whereas if the script failed to run at the `2_run_md_ssfep` step, the job handling script needs to be edited. The job handling scripts for SSFEP are:

- `templates/ssfep/job_lig_md.tmpl`
- `templates/ssfep/job_prot_lig_md.tmpl`
- `templates/ssfep/job_dG.tmpl`

Typically the header portion of the job submission script requires editing. Please contact support@silcsbio.com if you need assistance.

6.2 2. I don't have a cluster but I have a GPU workstation. What can I do?

You may be able to practically run the SilcsBio software if your GPU workstation has sufficient resources. An appropriate workstation may have at least 24 CPU cores, 4 GPUs, 64 GB of RAM, and 10 TB of disk space. Installing a job queueing system, such as the Slurm Workload Manager, will allow the SilcsBio server software to run on the workstation.

The SilcsBio Workstation is a turn-key GPU workstation hardware+software solution developed by SilcsBio that comes with all necessary software pre-installed. The SilcsBio workstation has a quiet, sleek form factor for use in an office setting and comes ready to plug in to a standard wall electrical socket. Please contact info@silcsbio.com for details.

6.3 3. I compiled my GROMACS with MPI and my job is not running

Please contact us so we can repackage the files with the appropriate command using `mpirun` instead.

Alternatively, you may edit the job handling script to edit the GROMACS command.

For example, the `mdrun` command is specified at the top of `templates/ssfep/job_lig_md.tmpl` file:

```
mdrun="${GMXDIR}/gmx mdrun -nt $nproc"
```

You may edit this to

```
mdrun="mpirun -np $nproc ${GMXDIR}/gmx mdrun"
```

6.4 4. GROMACS on the head node does not run because the head node and compute node have different operating systems.

In this case, we recommend compiling GROMACS on the head node and compiling `mdrun` only on the compute node.

Building only `mdrun` can be done by supplying the `-DGMX_BUILD_MDRUN_ONLY=on` keyword to the `cmake` command in the build process. Once the `mdrun` program is built, place it in the same `$GMXDIR` folder. Now template files need to be edited to use the `mdrun` command properly on the compute node.

For example, the `mdrun` command is specified at the top of the `templates/ssfep/job_lig_md.tmpl` file:

```
mdrun="${GMXDIR}/gmx mdrun -nt $nproc"
```

You may edit this to

```
mdrun="${GMXDIR}/mdrun -nt $nproc"
```

6.5 5. I get the “error while loading shared libraries: libcudart.so.8.0: cannot open shared object file: No such file or directory” message during my setup.

If you encounter this error, the most likely reason is that GROMACS was compiled on a machine having a GPU whereas the current machine where the command is being executed does not have a GPU.

It may be possible that the necessary library is already available for the machine even though it does not have a GPU. So, check if the `libcudart.so` file exists on the current machine. The most likely place is `/usr/local/cuda/lib64`. If the file exists in that location, add that path to your `LD_LIBRARY_PATH` environment variable..

If the library is not available on the current machine, we recommend following FAQ #4 to compile GROMACS and `mdrun` separately.

6.6 6. I want to modify the force field and topology files for SILCS simulation

As an example, if there is the need to add extra bonds that are not present in the standard force field definitions, this is the procedure to make the necessary modifications. For example, some proteins contain two metals ions adjacent to each other, in which case it may be useful to place a bond connecting the ions. The protein 3bi0 has two Zn ions adjacent to each other, and adding such a bond is useful to restrain the distance between the ions to that in the crystal structure. Please refer to the GROMACS documentation regarding to modify the `.top` and `ffbonded.itp` files.

First, run the following command. This will copy the basic force field to and generate an initial topology file in `1_setup/`, allowing you to edit them.

```
$SILCSBIODIR/silcs/1_setup_silcs_boxes prot=<prot PDB>
```

Then edit the force field parameter file `1_setup/charmm36.ff/ffbonded.itp`.

If you want to modify the topology file (e.g. to add an explicit bond between the ions), copy `1_setup/<prot>_gmx.top.1.bak` to `1_setup/<prot>_gmx.top`. Then edit `<prot>_gmx.top` and add the desired bond between the two ions in the `[bond]` list.

Once the files are edited, re-run the `1_setup` command with the `skip_pdb2gmx=true` keyword. This will preserve your edits and create the necessary files to run the SILCS simulations.

```
$SILCSBIODIR/silcs/1_setup_silcs_boxes prot=<prot PDB> skip_
↪pdb2gmx=true
```

Once this completes, run the `$SILCSBIODIR/silcs/2a_run_gcmd` script to initiate your SILCS simulations.

6.7 7. I want to visualize FragMaps using MOE

FragMaps are in the MAP grid file format. However, this file format is not supported in MOE. To be able to display FragMaps in MOE, we provide a script to convert MAP format files to CNS format files, which are supported by MOE.

```
$SILCSBIODIR/utils/map_to_cns.sh prot=<protein PDB> mapsdir=  
→<FragMap directory>
```

The above command will convert all `.map` files in the `mapsdir` folder to `.cns` format files.

SILCS: SITE IDENTIFICATION BY LIGAND COMPETITIVE SATURATION

7.1 Background

The design of small molecules that bind with optimal specificity and affinity to their biological targets, typically proteins, is based on the idea of complementarity between the functional groups in a small molecule and the binding site of the target. Traditional approaches to ligand identification and optimization often employ the one binding site/one ligand approach. While such an approach might be straightforward to implement, it is limited by the resource-intensive nature of screening and evaluating the affinity of large numbers of diverse molecules.

Functional group mapping approaches have emerged as an alternative, in which a series of maps for different classes of functional groups encompass the target surface to define the binding requirements of the target. Using these maps, medicinal chemists can focus their efforts on designing small molecules that best match the maps.

Site Identification by Ligand Competitive Saturation (SILCS) offers rigorous free energy evaluation of functional group affinity pattern for the entire 3D space in and around a protein [5]. The SILCS method yields functional group free energy maps, or FragMaps, which are precomputed and then used to rapidly facilitate ligand design. FragMaps are generated by molecular dynamics (MD) simulations that include protein flexibility and explicit solvent/solute representation, thus providing an accurate, detailed, and comprehensive set of data that can be used in database screening, fragment-based drug design, and lead optimization of small molecules.

In the context of biological therapeutics, the comprehensive nature of the FragMaps is of utility for excipient design as all possible binding sites of all possible excipients and buffers can be identified and quantified.

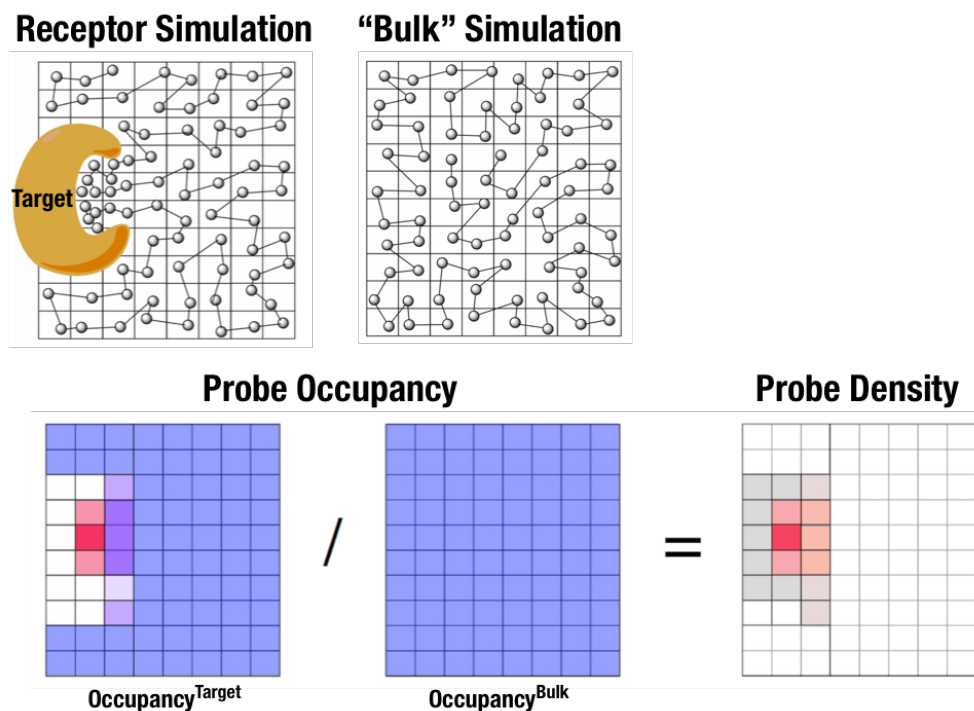


Fig. 7.1: Illustration of fragment density maps.

Fig. 7.1 illustrates FragMaps generation. Two MD simulations of a probe molecule (e.g., benzene) are performed in the presence of a protein and in aqueous solution (without protein), resulting in two occupancy maps, O^{target} and O^{bulk} , respectively. The GFE (grid free energy) FragMaps are then derived by the following formula

$$\text{GFE}_{x,y,z}^T = -RT \log \frac{O_{x,y,z}^{\text{target}}}{\langle O^{\text{bulk}} \rangle}$$

By generating FragMaps using various small solutes representing different functional groups including benzene, propane, methanol, imidazole, formamide, acetaldehyde, methylammonium, acetate, and water, it is possible to map the functional group affinity pattern of a protein. FragMaps encompass the entire protein such that all FragMap types are in all regions. Thus, information on the affinity of all the different types of functional groups is available in and around the full 3D space of the protein or other target molecule.

FragMaps may be used in a qualitative fashion to facilitate ligand design by allowing the medicinal chemist to readily visualize regions where the ligand can be modified or functional groups added to improve affinity and specificity. As the FragMaps include protein flexibility, they indicate regions of the target protein that can “open” thereby identifying regions under the protein surface accessible for ligand binding. In addition to FragMaps, an “exclusion map” is generated based on regions of the system that are not sampled at all by water or probe molecules during the MD simulations. Therefore, the exclusion map represents a strictly inaccessible surface.

Many proteins contain binding sites that are partially or totally inaccessible to the surrounding solvent environment that may require partial unfolding of the protein for ligand binding to occur.

SILCS sampling of such deep or inaccessible pockets is facilitated by the use of a Grand Canonical Monte Carlo (GCMC) sampling technique in conjunction with MD simulations [6]. Thus, the SILCS technology is especially well-suited for targeting the deep and inaccessible binding sites found in targets like GPCRs and nuclear receptors.

Once a set of ligand-independent SILCS FragMaps are produced, they can be used for various purposes that rapidly rank ligand binding in a highly computationally efficient manner for multiple ligands **WITHOUT** recalculating the FragMaps. Applications of SILCS methodology include:

- Binding site identification
 - Binding pocket searching with known ligands
 - Binding pocket identification via pharmacophore generation
 - Binding pocket identification via fragment screening
- Database screening
 - SILCS 3D pharmacophore models
 - Ligand posing using available techniques (Catalyst, etc)
 - Ligand ranking
- Fragment-based ligand design
 - Identification of fragment binding sites
 - Estimation of ligand affinity following fragment linking
 - Expansion of fragment types
- Ligand optimization
 - Qualitative ligand optimization by FragMaps visualization
 - Quantitative evaluation of ligand atom contributions to binding
 - Quantitative estimation of relative ligand affinities
 - Quantitative estimation of large numbers of ligand chemical transformations

SILCS generates 3D maps of interaction patterns of functional group with your target molecule, called FragMaps. This unique approach simultaneously uses multiple different small solutes with various functional groups in explicit solvent MD simulations that include target flexibility to yield 3D FragMaps that encompass the delicate balance of target-functional group interactions, target desolvation, functional group desolvation and target flexibility. The FragMaps may then be used to both qualitatively direct ligand design and quantitatively to rapidly evaluate the relative affinities of large numbers of ligands following the precomputation of the FragMaps.

This chapter goes over the workflow of generating and visualizing FragMaps.

7.2 Running SILCS simulations from the SilcsBio GUI

Please see *SILCS simulation from the GUI* as described in *Graphical User Interface Quickstart* for a step-by-step description for using the SilcsBio GUI for SILCS simulations.

FragMap generation using SILCS begins with a well-curated protein structure file (without ligand) in PDB file format. We recommend keeping only those protein chains that are necessary for the simulation, removing all unnecessary ligands, renaming non-standard residues, filling in missing atomic positions, and, if desired, modeling in missing loops. Please contact support@silcsbio.com if you need assistance with protein preparation.

A typical SILCS simulation can produce output totaling in excess of 100 GB, so please select a folder with appropriate free space.

The setup process automatically builds the topology of the simulation system, creating metal-protein bonds if metal ions are present, rotates side chain orientations to enhance sampling, and places probe molecules around the protein. The SilcsBio GUI provides Advanced Settings options prior to the setup process and prior to running the SILCS simulations.

The screenshot displays the 'SILCS Simulation Setup' window. On the left is a sidebar with 'Setup', 'Simulation', and 'Fragmap' options. The main area contains the following fields and controls:

- Project Name:** 3fly_silcs
- Remote Server:** silcsserver
- Protein PDB File:** 3fly_fixed.pdb
- Advanced Settings:**
 - Project Location:** Project will be stored in \$HOME/silcsbio/projects
 - Use Existing Setup:** select setup folder
 - Use Existing Trajectory:** select trajectory folder
 - Use Scramble SC:** ☒
 - Number of Systems:** 8
- Buttons:** Setup, Reset

A red arrow points to a small downward arrow icon on the right side of the Advanced Settings section, with the text 'Click to display/hide "Advanced Settings"'.

Tip: Default settings have been optimized for SILCS FragMap simulation. If you do wish to change the number of simulations to run or to run simulations with a different number of CPUs, you can make adjustments in the Advanced Settings options.

Tip: If a simulation stops prematurely, the progress bar will turn red and show a “Restart” option.

When the job is restarted, the simulation will continue from the last cycle, but the progress bar may reset. This is because job recovery iterates over existing cycles to check if output was correctly produced. The completed cycles will be automatically skipped and the progress will update to where the job had stopped. That is to say, restarting a failed or stopped job will *not* cause you to lose existing job progress.

7.3 Running SILCS simulations from the command line interface

Please see *SILCS simulation from the command line* as described in *Command Line Interface Quickstart* for a step-by-step description for using the Command Line Interface (CLI) for SILCS simulations.

FragMap generation using SILCS begins with a well-curated protein structure file (without ligand) in PDB file format. We recommend keeping only those protein chains that are necessary for the simulation, removing all unnecessary ligands, renaming non-standard residues, filling in missing atomic positions, and, if desired, modeling in missing loops. Please contact support@silcsbio.com if you need assistance with protein preparation.

7.3.1 SILCS simulation setup

```
${SILCSBIODIR}/silcs/1_setup_silcs_boxes prot=<Protein PDB>
```

Warning: The setup program internally uses the GROMACS utility `pdb2gmx`, which may have problems processing the protein PDB file. The most common reasons for errors at this stage involve mismatches between the expected residue name/atom names in the input PDB and those defined in the CHARMM force-field.

To fix this problem: Run the `pdb2gmx` command manually from within the `1_setup` directory for a detailed error message. The setup script already prints out the exact commands you need to run to see this message.

```
cd 1_setup
pdb2gmx -f <PROT PDB NAME>_4pdb2gmx.pdb -ff charmm36 -water tip3p -o test.pdb -p test.top -ter -merge all
```

Once all the errors in the input PDB are corrected, rerun the setup script.

Following completion of the setup, run 10 GCMC/MD jobs using the following script:

```
${SILCSBIODIR}/silcs/2a_run_gcmd prot=<Protein PDB>
```

This script will submit 10 jobs to the predefined queue. Each job runs out 100 ns of GCMC/MD with the protein and the solute molecules.

Once the simulations are complete, the `2a_run_gcmd/[1-10]` directories will contain `*.prod.125.rec.xtc` trajectory files. If these files are not generated, then your simulations are either still running or stopped due to a problem. Look in the log files within these directories to diagnose problems.

7.3.2 FragMap generation

Once your simulations are done, generate the FragMaps using the following script.

```
${SILCSBIODIR}/silcs/2b_gen_maps prot=<Protein PDB>
```

This script will submit 10 single-core jobs. These will build occupancy maps spanning the simulation box for selected solute molecule atoms representing different functional groups. For a ~50K atom simulation system, this step takes approximately 10-20 minutes to complete. The FragMaps have a default spacing of 1 Å.

The next step is to combine the occupancy FragMaps generated from individual simulations and convert them into GFE FragMaps. Each voxel in a GFE FragMap contains a Grid Free Energy (GFE) value for the probe type that was used to create the corresponding occupancy FragMap.

```
${SILCSBIODIR}/silcs/2c_fragmap prot=<Protein PDB>
```

GFE FragMaps will be placed in the `silcs_fragmap_<protein PDB>/maps` directory. PyMOL and VMD scripts to loads these file will be placed in the `silcs_fragmap_<protein PDB>` directory.

<code><prot name>.benc.gfe.map</code>	Aromatic map
<code><prot name>.prpc.gfe.map</code>	Aliphatic map
<code><prot name>.aceo.gfe.map</code>	Charged acceptor map
<code><prot name>.mamn.gfe.map</code>	Charged donor map
<code><prot name>.forn.gfe.map</code>	Polar nitrogen donor map (Formamide)
<code><prot name>.foro.gfe.map</code>	Polar oxygen acceptor map (Formamide)
<code><prot name>.imin.gfe.map</code>	Polar nitrogen acceptor map (Imidazole)
<code><prot name>.iminh.gfe.map</code>	Polar nitrogen donor map (Imidazole)
<code><prot name>.aalo.gfe.map</code>	Polar oxygen acceptor map (Acetaldehyde)
<code><prot name>.meoo.gfe.map</code>	Polar oxygen acceptor map (Methanol)
<code><prot name>.apolar.gfe.map</code>	Generic Apolar maps
<code><prot name>.hbdon.gfe.map</code>	Generic donor maps
<code><prot name>.hbacc.gfe.map</code>	Generic acceptor maps
<code><prot name>.excl.map</code>	Exclusion map
<code><prot name>.ncla.map</code>	Zero map

7.3.3 Cleanup

Raw trajectory files are large. To reduce file sizes, hydrogen atom positions can be deleted with the following:

```
${SILCSBIODIR}/silcs/2d_cleanup prot=<Protein PDB>
```

7.4 SILCS simulation setup with GPCR targets

You may use either a bare protein structure or your own pre-built protein/bilayer system with SILCS.

If you are beginning with a bare protein, SilcsBio provides a command line utility to embed trans-membrane proteins, such as GPCRs, in a bilayer of 9:1 POPC/Cholesterol for subsequent SILCS simulations:

```
${SILCSBIODIR}/silcs-memb/1a_fit_protein_in_bilayer prot=<Protein PDB>
```

This command will align the first principal axis of the protein with the bilayer normal (Z-axis) and translate the protein center of the mass to the center of the bilayer (Z=0).

If the protein is already oriented as desired, `orient_principal_axis=false` will suppress automatic principal axis-based alignment.

```
${SILCSBIODIR}/silcs-memb/1a_fit_protein_in_bilayer prot=<Protein PDB>
→orient_principal_axis=false
```

By default the system size is set to 120 Å along the X and Y dimensions. To change the system size, use the `bilayer_x_size` and `bilayer_y_size` options.

```
${SILCSBIODIR}/silcs-memb/1a_fit_protein_in_bilayer prot=<Protein PDB>
→bilayer_x_size=<X dimension> bilayer_y_size=<Y dimension>
```

The resulting protein/bilayer system will be output in a PDB file with suffix `_popc_chol`. It is important to use a molecular visualization software at this stage to confirm correct orientation of the protein and size of the bilayer before using this output as the input in the next step.

Alternatively, you may use a protein/bilayer system that has been built using other software tools.

The following command will prepare the SILCS simulation box by adding water and probe molecules to the protein/bilayer system:

```
${SILCSBIODIR}/silcs-memb/1b_setup_silcs_with_prot_bilayer prot=
→<Protein/bilayer PDB>
```

SILCS GCMC/MD simulation can now be launched with the following command:

```
{{SILCSBIODIR}}/silcs/2a_run_gcmd prot=<Protein/bilayer PDB> memb=true
```

The SILCS GCMC/MD will begin with a 6-step pre-equilibration to slowly relax the bilayer followed by 10 ns of relaxation of the protein into the bilayer prior to the production simulation.

Tip: GPCR simulation systems are typically large and can require significant storage space (> 200 GB) and simulation time (> 14 days with GPUs).

FragMap generation from the GCMC/MD simulation data follows the same protocol as for non-bilayer systems. Refer to previous instructions for `{{SILCSBIODIR}}/silcs/2b_gen_maps` and `{{SILCSBIODIR}}/silcs/2c_fragmap`.

If you encounter a problem, please contact support@silcsbio.com.

7.5 SILCS simulation setup with halogen probes

SILCS probes with halogen-containing functional groups can be useful for extending the diversity of chemical space covered by FragMaps. While a common approach is to consider halogen atoms as non-polar groups, research on non-covalent “halogen bonding” suggests halogen atoms warrant special treatment.

The CHARMM General Force Field CGenFF v.2.3.0+ allows halogen atoms to be treated with lonepairs to achieve directionality in polar interactions. These force field improvements are included in SILCS simulations from version 2020.1 of the SilcsBio software, and halogen-protein interactions can be determined using a set of halogenated probes in SILCS simulations.

To include halogenated probes in your simulation, add the `halogen=true` keyword:

```
{{SILCSBIODIR}}/silcs/1_setup_silcs_boxes prot=<Protein PDB>
→halogen=true
{{SILCSBIODIR}}/silcs/2a_run_gcmd prot=<Protein PDB> halogen=true
{{SILCSBIODIR}}/silcs/2b_gen_maps prot=<Protein PDB> halogen=true
{{SILCSBIODIR}}/silcs/2c_fragmaps prot=<Protein PDB> halogen=true
```

This will create folders ending with `_x` (i.e., `1_setup_x/`, etc.) to denote SILCS-Halogen simulation systems, as opposed to standard SILCS simulations. While the SilcsBio GUI does not currently support running such jobs, it will automatically load FragMaps from these simulations alongside standard FragMaps.

If you encounter a problem, please contact support@silcsbio.com.

7.6 Resuming stopped SILCS jobs

Situations such as exceeding workload manager (job queue) walltime limits can cause SILCS jobs to stop before running to completion. Resuming such jobs from where they stopped is straightforward. On the server where the job was running, go to the directory `<Project Location>/2a_run_gcmd/i`, where `i` is an integer from 1 to 10 and corresponds to the stopped job among the ten SILCS GCMC/MD jobs that were being run for that particular target. In that directory, directly use the file `job_mc_md.cmd` to submit the job to the workload manager, for example `qsub job_mc_md.cmd` for Sun Grid Engine (SGE) and `sbatch job_mc_md.cmd` for Slurm.

To ensure a successful restart, make sure to issue any required extra setup commands (for example, as listed in the “Extra setup” field under *Settings and remote server configuration* from the SilcsBio GUI Home screen) such as `export LD_LIBRARY_PATH=<path/to/cuda/libraries>` or `module load <name of cuda module>` before submitting the job.

7.7 Visualizing FragMaps

GFE FragMaps are created in the directory `2b_gen_maps`. This folder contains the specific and generic FragMaps listed previously. FragMaps are defined using on non-hydrogen atoms. For certain solute types there are multiple FragMaps (imidazole, formamide). FragMaps for water oxygens (tipo) are included to identify water molecules that are difficult to displace. Generic FragMaps for apolar (benzene, propane), H-bond donor (neutral H-bond donors) and H-bond acceptor (neutral H-bond acceptors) probe types are included to simplify visualization.

The easiest way to visualize FragMaps is with the SilcsBio GUI. SilcsBio also provide plugins for the visualization softwares VMD and PyMOL:

7.7.1 FragMaps in VMD

Please ensure the SilcsBio VMD plugin is installed before continuing (see [VMD plugin installation](#)).

First, load the protein structure used for FragMap generation. Once the protein structure is loaded, select *Extensions* → *SilcsBio* → *FragMap Tools* to activate the plugin window.

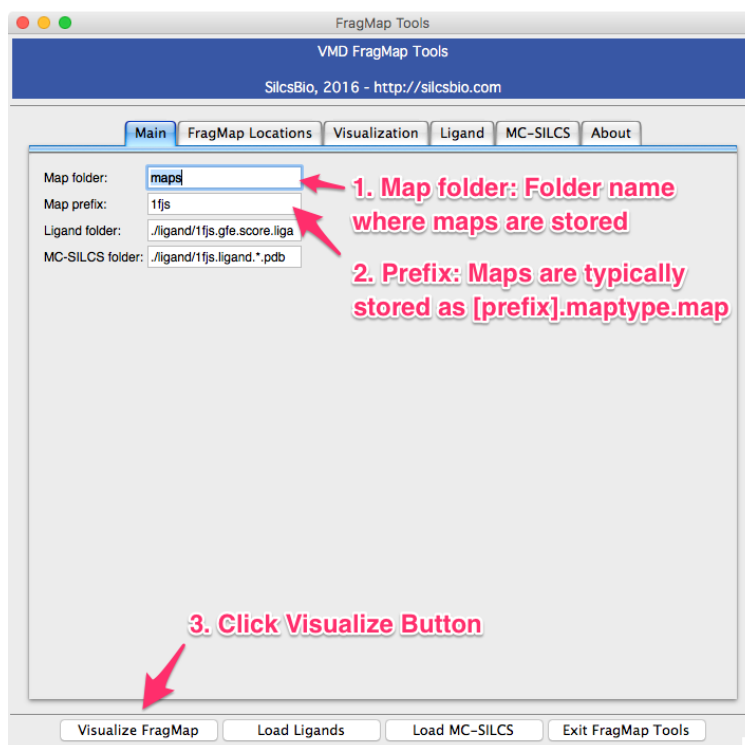
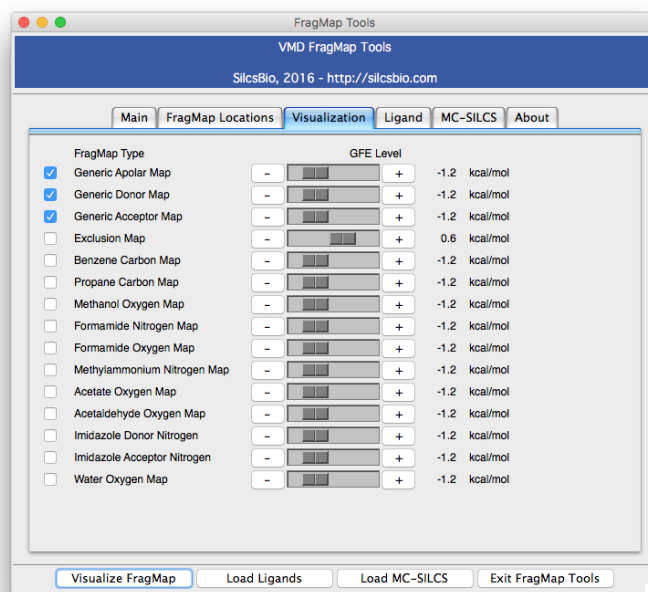


Fig. 7.2: VMD FragMap Plugin

“Map folder” is the name of the folder that contains FragMap files, and is typically “2b_gen_maps”. “Map prefix” is the protein PDB name used when setting up and performing the SILCS simulations. If a protein PDB file was loaded in to VMD prior to opening the plugin window, the name of the PDB file will be taken as a default for this parameter. Once these two parameters are confirmed, click the “Visualize FragMap” button at the bottom of the window.

At this point, FragMaps will have been loaded in to the VMD Main window and a new tab will have appeared in the plugin window. This new tab allows quick enabling/disabling of FragMap display using checkboxes on the left hand side of the window. By default, GFE FragMaps are displayed at a contour level of -1.2 kcal/mol. This level can be changed by using either the +/- buttons or the slider.



7.7.2 FragMaps in PyMOL

Please ensure the SilcsBio PyMol plugin is installed before continuing (see *PyMOL plugin installation*).

First, load the protein structure used for FragMap generation. Once the protein structure is loaded, select *Plug* → *FragMap Tools* to activate the plugin window.

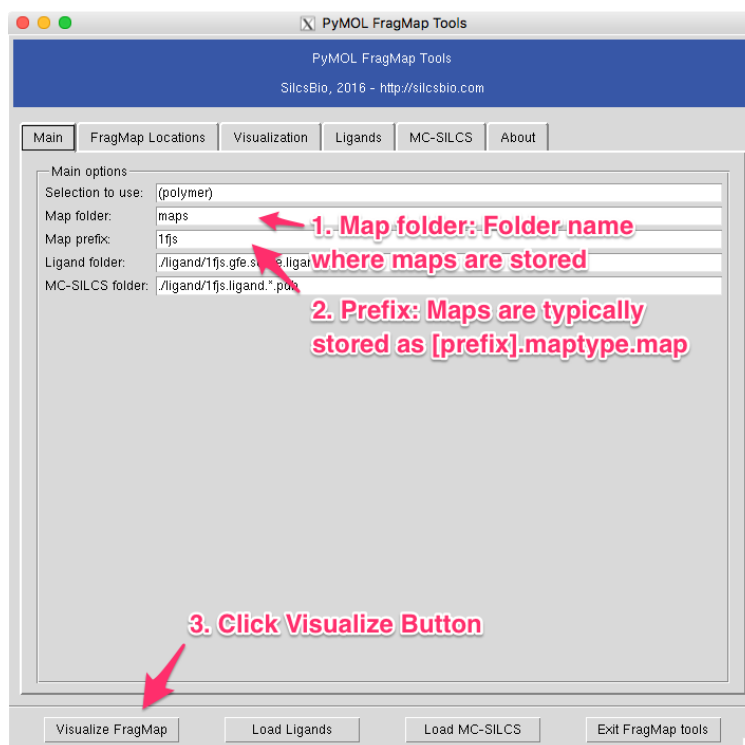
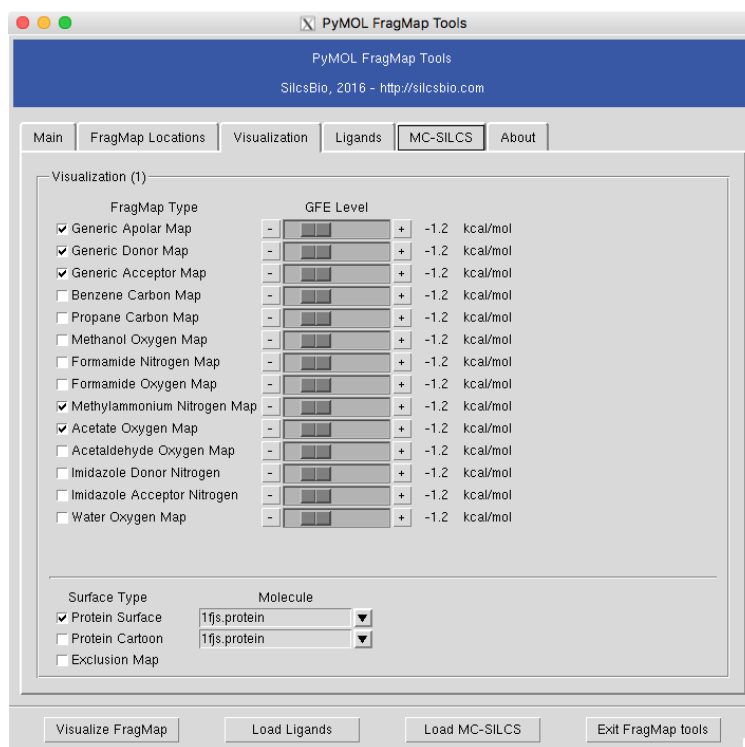


Fig. 7.3: PyMOL FragMap Plugin

“Map folder” is the name of the folder that contains FragMap files, and is typically “2b_gen_maps”. “Map prefix” is the protein PDB name used when setting up and performing the SILCS simulations. If a protein PDB file was loaded in to PyMol prior to opening the plugin window, the name of the PDB file will be taken as a default for this parameter. Once these two parameters are confirmed, click the “Visualize FragMap” button at the bottom of the window.

At this point, FragMaps will have been loaded in to the Pymol main window and a new tab will have appeared in the plugin window. This new tab allows quick enabling/disabling of FragMap display using checkboxes on the left hand side of the window. By default, GFE FragMaps are displayed at a contour level of -1.2 kcal/mol (with the exception of the Exclusion Map). This level can be changed by using either the +/- buttons or the slider.



SILCS-MC: LIGAND OPTIMIZATION

8.1 Background

The power of SILCS lies in the ability to use FragMaps to rapidly evaluate binding of diverse ligands to a target. SILCS-MC is Monte-Carlo (MC) sampling of ligands in translational, rotational, and torsional space in the field of FragMaps. MC sampling uses CGenFF intramolecular energies and the Ligand Grid Free Energy (LGFE), which is the sum of atomic Grid Free Energies (GFEs). The Exclusion Map prevents ligand sampling where no probe or water molecules visited during SILCS simulations. SILCS-MC allows for rapid conformational sampling of the ligand while accounting for protein flexibility in a mean-field-like fashion since ligand affinity and volume exclusion are embedded in the combination of FragMaps and the Exclusion Map. Final ligand scoring is based on the LGFE score [10][11].

FragMaps previously generated for a target (see *SILCS: Site Identification by Ligand Competitive Saturation*) can be used for optimization of a parent ligand by rapidly evaluating LGFE scores for derivatives of the parent ligand. The SILCS-MC approach has two principal advantages over free energy perturbation (FEP): functional group modifications to the parent ligand are very quick to evaluate, and there is no upper size limit on these modifications. Because the calculations are done in the context of pre-computed FragMaps, it is easy to decide which functional groups are candidates for modification simply by viewing the binding pose of the parent along with the FragMaps. FragMap densities adjacent to but unoccupied by parent ligand atoms offer opportunities for growing the parent ligand. Conversely, parent ligand atoms not in favorable regions of the FragMap densities can be candidates for modification or deletion without affecting binding affinity. Quantitative evaluation of individual functional group contributions to binding affinity can be achieved by analysis of atomic GFE scores. These capabilities are particularly useful when modifying functional groups to optimize pharmacokinetic properties or attempting to reduce the ligand size while maintaining affinity.

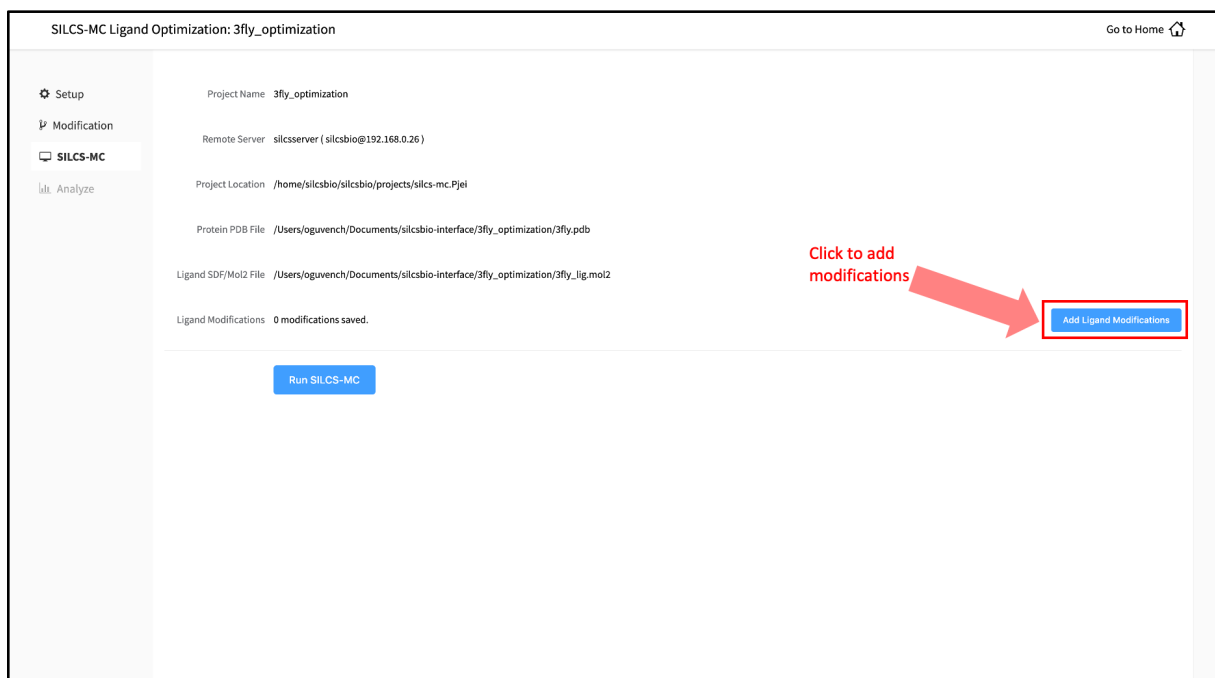
8.2 Running SILCS-MC ligand optimization from the SilcsBio GUI

1. Select *New SILCS-MC Optimization project* from the Home page.
2. Enter a project name and select the remote server where your SILCS-MC jobs will run. Next, provide FragMap, protein, and ligand input files. You may choose these files from the computer where you are running the SilcsBio GUI (“localhost”) or from any server you have previously configured, as described in *File and directory selection*.

The screenshot displays the 'SILCS-MC Ligand Optimization' web interface. On the left is a sidebar with navigation links: 'Setup' (active), 'Modification', 'SILCS-MC', and 'Analyze'. The main content area is titled 'SILCS-MC Ligand Optimization' and includes a 'Go to Home' link. The 'Setup' form contains the following elements: a text input for 'Project Name' with the value '3fly_optimization'; a dropdown menu for 'Remote Server' set to 'silcsserver'; a 'FragMap Location' field showing 'downloading...' with a close icon; two file selection buttons, 'select file' for 'Protein PDB File' and 'select ligand file' for 'Reference Ligand File'; an 'Advanced Settings' section with a right-pointing arrow; and two buttons at the bottom, 'Setup' and 'Reset'.

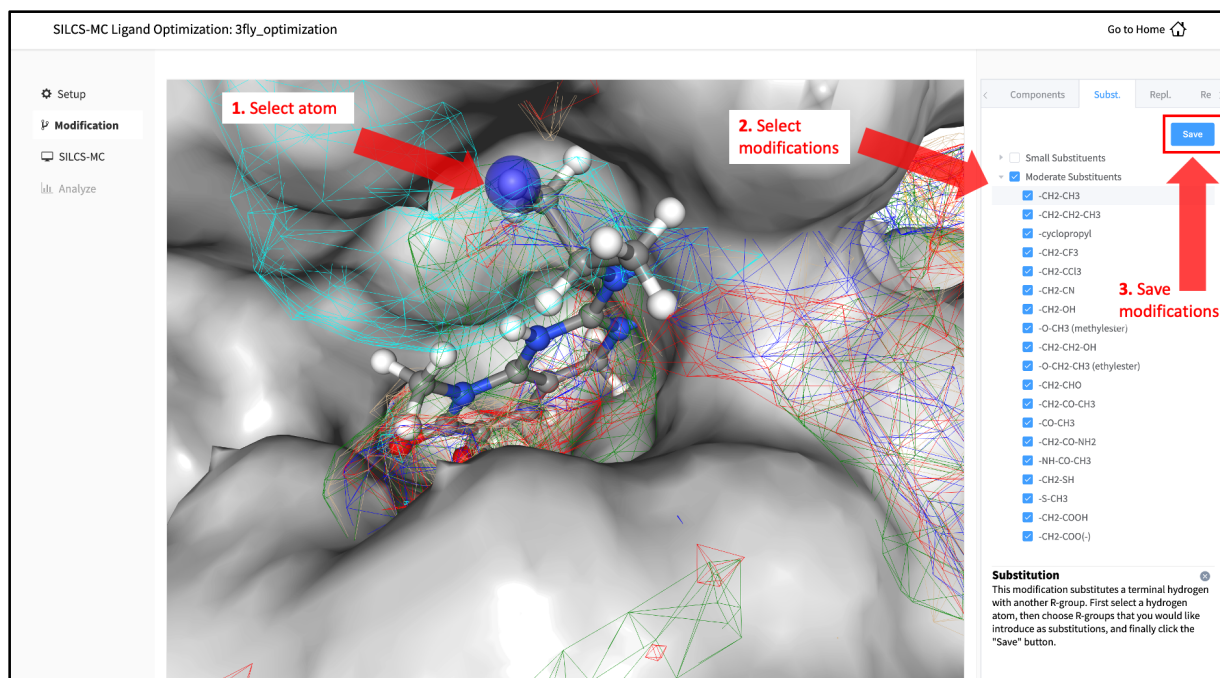
Tip: The “Reference Ligand File” must be an SD or Mol2 format file that contains the parent ligand aligned in the binding pocket of the input protein.

3. Once all information is entered correctly, press the “Setup” button at the bottom of the page. The GUI will contact the remote server and upload the your input files to the “Project Location” directory on the remote server. A green “Setup Successful” button will appear once the upload has successfully completed. Press this button to proceed.
4. The GUI will display a summary screen with the Project Name, Remote Server, Project Location, Protein PDB File, Ligand SDF/Mol2 File, and Ligand Modifications. The entry for Ligand Modifications will state “0 modifications saved” and have a button labeled “Add Ligand Modifications.” Press this button to select modifications to the parent ligand for evaluation by SILCS-MC.



- You will now see the parent ligand in the binding pocket. Rotate the view until you can easily see the functional group you wish to modify. There are two major modification types, Substitution and Replacement, available in the GUI. Substitution is used to substitute an atom with a functional group. Replacement is used to replace an atom in a ring or aliphatic acyclic group with another functional group that preserves the connectivity and valence of the ring or chain.

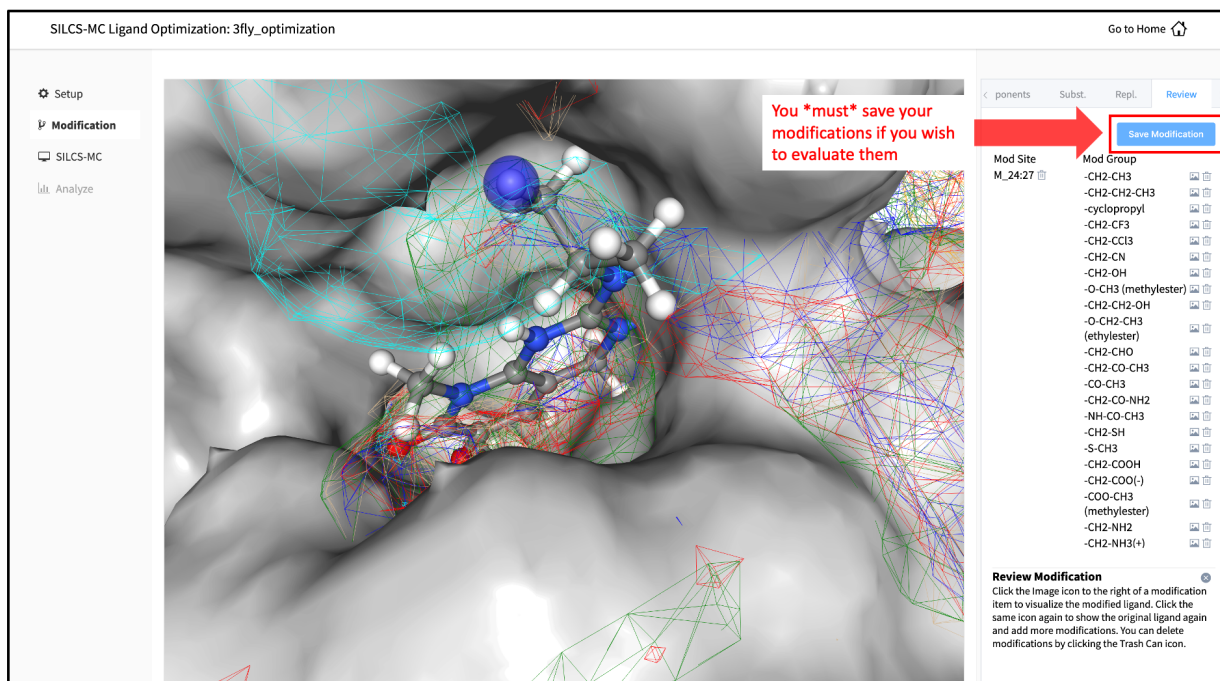
In the visualization window, select the atom to be modified. Then, select your desired modifications from the “Substitution” or the “Replacement” tab in the right-hand panel. Pressing the “Save” button in the panel will take you to the “Review” tab.



The list of modification types in the GUI covers a very broad range of chemical functionality and size.

6. Use the “Review” tab to confirm your desired modifications.

Valid modifications will have a small Image icon as well as a small Trash Can icon. Clicking on the Image icon will show the modification in the center panel. Clicking on it again will show the parent ligand. Clicking on the Trash Can icon will delete the proposed modification from your list. You can go back to the “Substitution” and “Replacement” tabs to add to your list. Once you have completed your list of modifications, **you must press the “Save Modification” button in the “Review” tab to actually save the list of modifications for evaluation by SILCS-MC.**



- Once you have saved all your desired modifications, click on *SILCS-MC* in the left-hand panel to go back to the summary screen. The “Ligand Modifications” will have been updated to reflect the number of saved modifications that will be evaluated by SILCS-MC. Because SILCS-MC is a fast calculation, it is feasible to select a very large number of modifications (hundreds+) for quick evaluation in a single run.

SILCS-MC Ligand Optimization: 3fly_optimization

Go to Home

Setup
Modification
SILCS-MC
Analyze

Project Name: 3fly_optimization

Remote Server: silcsserver (silcsbio@192.168.0.26)

Project Location: /home/silcsbio/silcsbio/projects/silcs-mc.Pjei

Protein PDB File: /Users/oguvench/Documents/silcsbio-interface/3fly_optimization/3fly.pdb

Ligand SDF/Mol2 File: /Users/oguvench/Documents/silcsbio-interface/3fly_optimization/3fly_lig.mol2

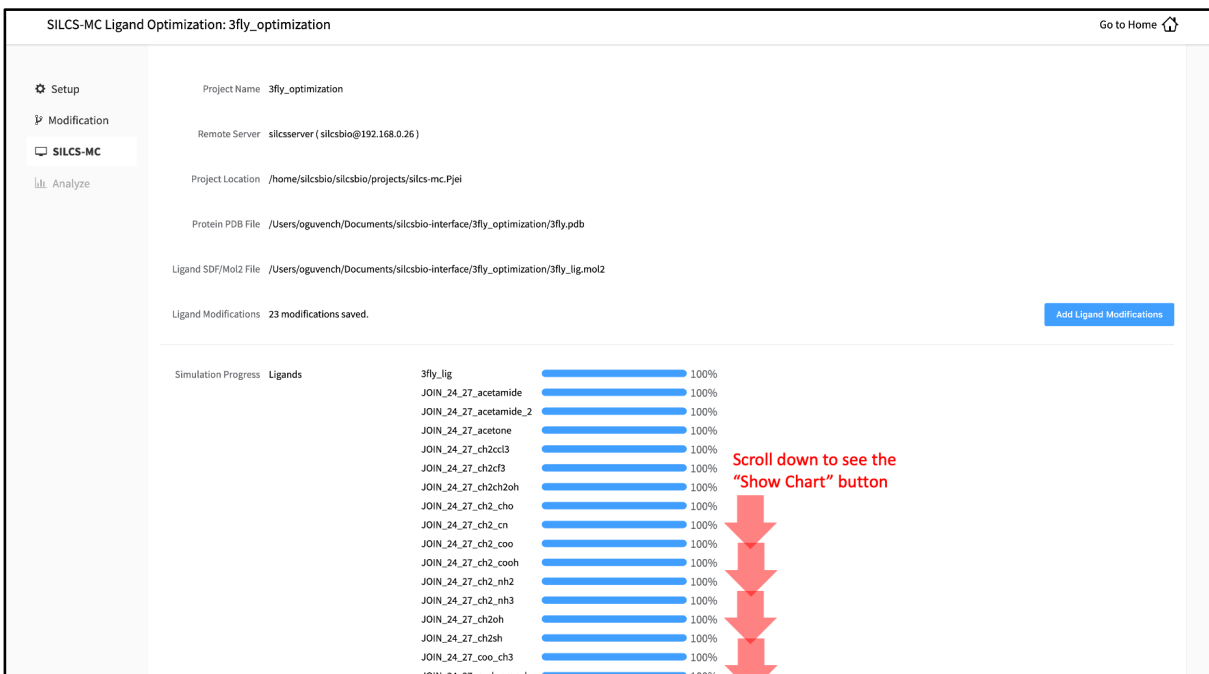
Ligand Modifications: 23 modifications saved.

Add Ligand Modifications

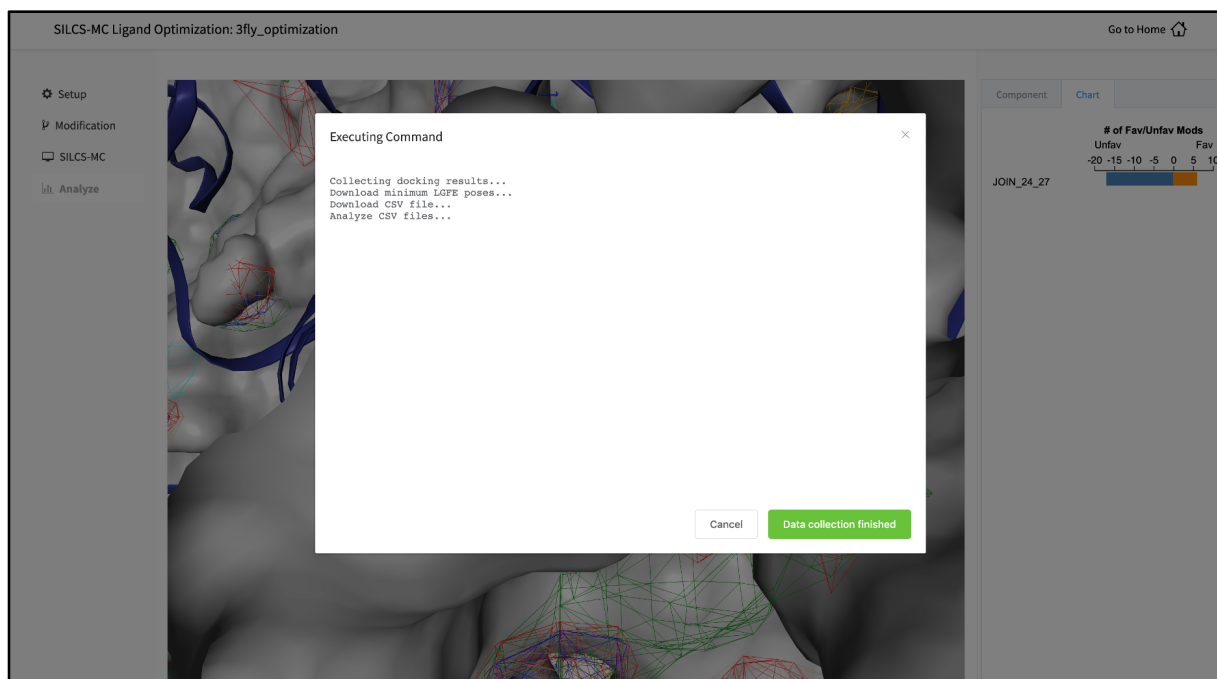
Run SILCS-MC

Click the “Run SILCS-MC” button to launch your jobs on the remote server.

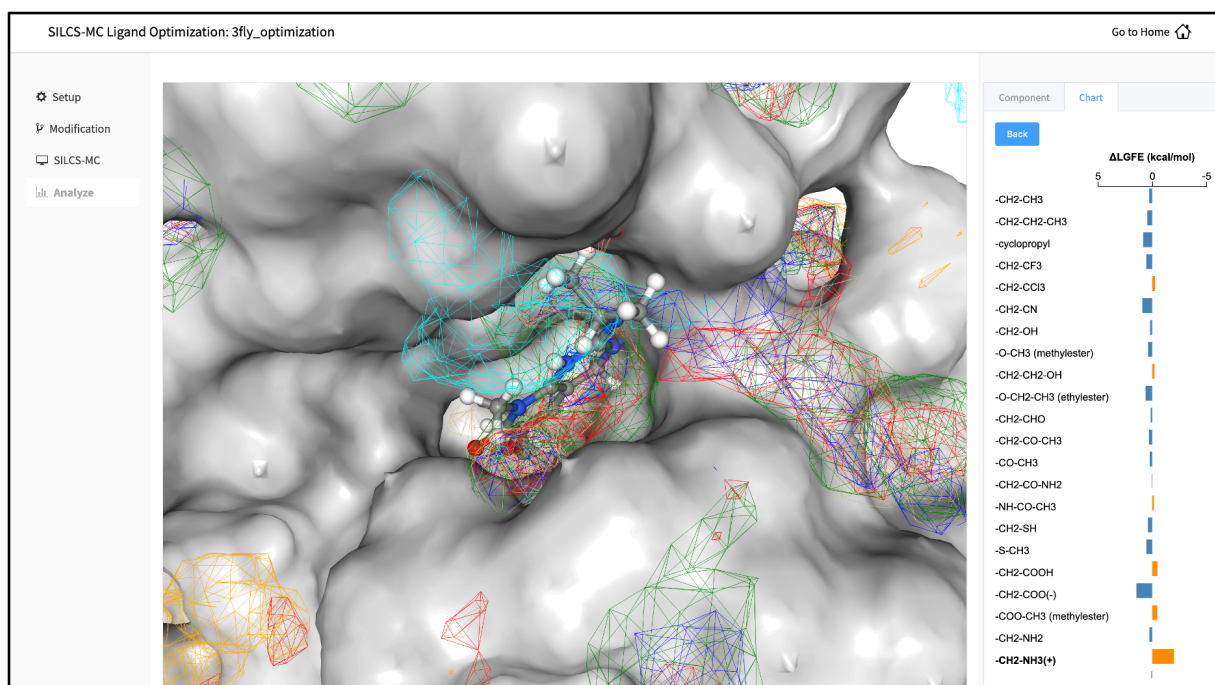
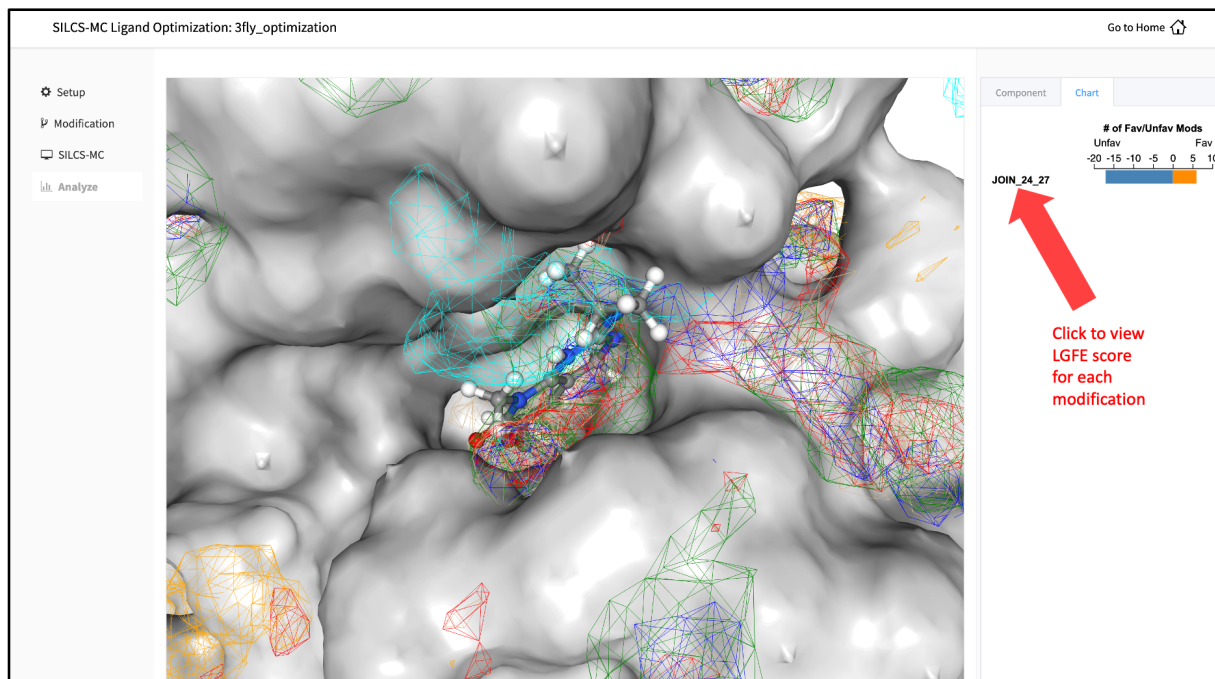
You can monitor job progress in the SilcsBio GUI, and click on the “Show Chart” button once all job progress bars are at 100%.



- Clicking the “Show Chart” button after jobs have reached 100% will download the SILCS-MC results from the server. Click on “Data collection finished” to proceed.



- The “Chart” tab in the right-hand panel shows a summary of the number of favorable and unfavorable modifications associated with a particular modification site. Click on the name of the modification site in that panel to see a detailed list of the change in Ligand Grid Free Energy ($\Delta LGFE$) relative to the parent ligand for each modification at that site.



Clicking on the name of a modification will display that modification in the central panel.

SILCS-MC: DOCKING AND POSE REFINEMENT

9.1 Background

The power of SILCS lies in the ability to use FragMaps to rapidly evaluate binding of diverse ligands to a target. SILCS-MC is Monte-Carlo (MC) sampling of ligands in translational, rotational, and torsional space in the field of FragMaps. MC sampling uses CGenFF intramolecular energies and the Ligand Grid Free Energy (LGFE), which is the sum of atomic Grid Free Energies (GFEs). The Exclusion Map prevents ligand sampling where no probe or water molecules visited during SILCS simulations. SILCS-MC allows for rapid conformational sampling of the ligand while accounting for protein flexibility in a mean-field-like fashion since ligand affinity and volume exclusion are embedded in the combination of FragMaps and the Exclusion Map. Final ligand scoring is based on the LGFE score [10][11].

SILCS-MC can be used to generate and score binding poses for a ligand to a target using SILCS FragMaps that have been previously computed for that target (see *SILCS: Site Identification by Ligand and Competitive Saturation*). This can readily be done for a single ligand or a database of ligands. Two default conformational sampling protocols are available, “docking” and “pose refinement,” as described in detail below. The docking protocol is useful when no information is available about the binding pose, as it entails extensive translational, rotational, and intramolecular conformational sampling. The pose refinement protocol is useful when a reasonable starting pose for the ligand is available, for example to re-score poses output by another high-throughput in silico screening tool. Instructions for developing custom SILCS-MC ligand conformational sampling protocols are provided at the end of this chapter.

9.2 Running SILCS-MC docking

Docking is exhaustive sampling of a ligand’s conformation in a given pocket to determine its most favorable orientation and internal geometry as defined by LGFE scoring. The pocket is predefined as a 10 Å sphere and the center of the pocket is taken as either the center of the supplied ligand molecule coordinates or explicitly given by the user. This protocol entails five independent MC runs with the ligand.

This protocol is recommended for ligands with diverse chemotypes and unknown binding poses. When the pose of a parent ligand is known and SILCS-MC evaluations are to be performed over a congeneric series, the pose refinement protocol is recommended instead (see below).

9.2.1 Running SILCS-MC docking from the SilcsBio GUI

1. Select *New SILCS-MC Docking project* from the Home page.
2. Enter a project name and select the remote server where the SILCS-MC docking jobs will run. Also, provide FragMap and protein input files. You may choose these files from the computer where you are running the SilcsBio GUI (“localhost”) or from any server you have previously configured, as described in [File and directory selection](#). You will additionally need to provide a “Ligand SDF File” that contains the database of ligands to be docked.

SILCS-MC Docking Ligands

Close X

Setup

SILCS-MC

Analyze

Project Name 3fly_dck

Remote Server silcsserver

FragMap Location ...pt/7mw76b4s6lqctvb7z_fmccc0000gn/7/silcs_fragmaps_3fly/maps X

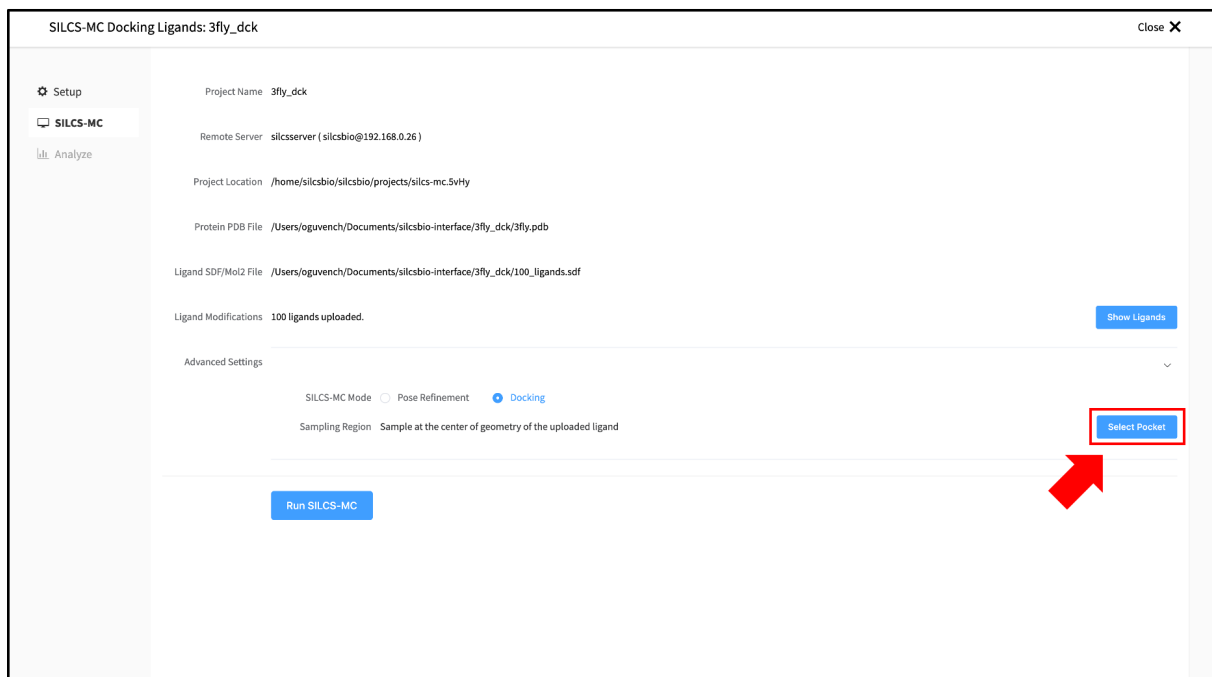
Protein PDB File ...mw76b4s6lqctvb7z_fmccc0000gn/7/silcs_fragmaps_3fly/3fly.pdb X

Ligand File .../folders/pt/7mw76b4s6lqctvb7z_fmccc0000gn/7/100_ligands.sdf X

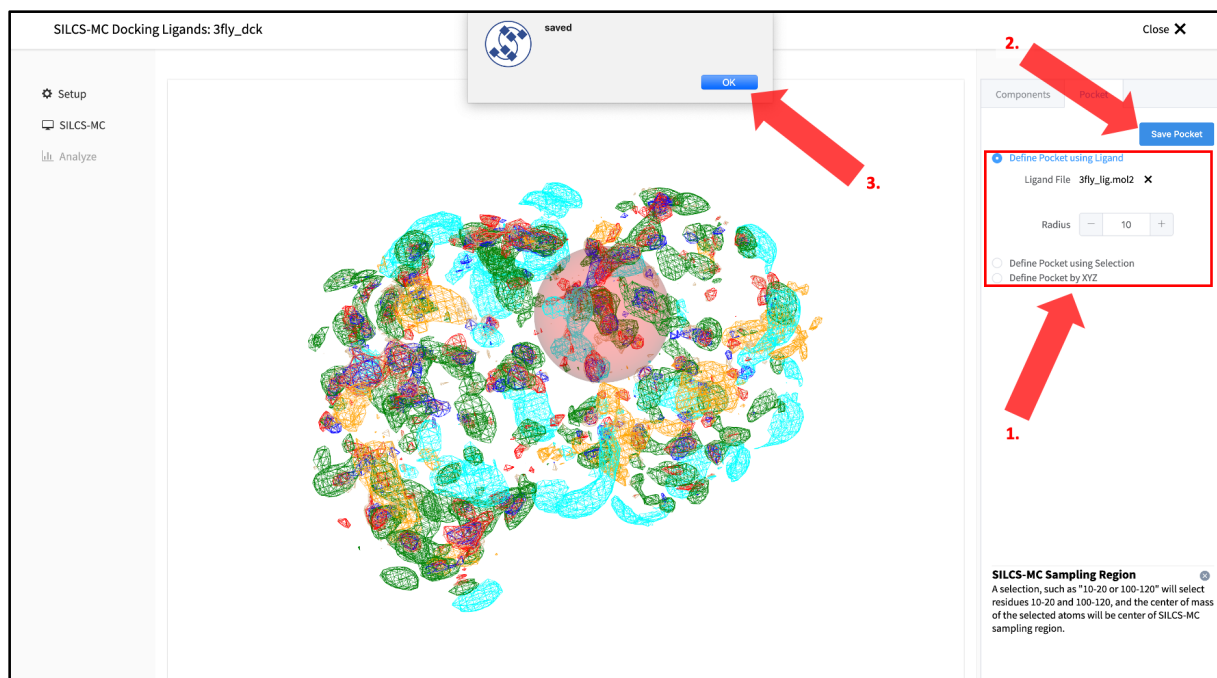
Advanced Settings >

Setup Reset

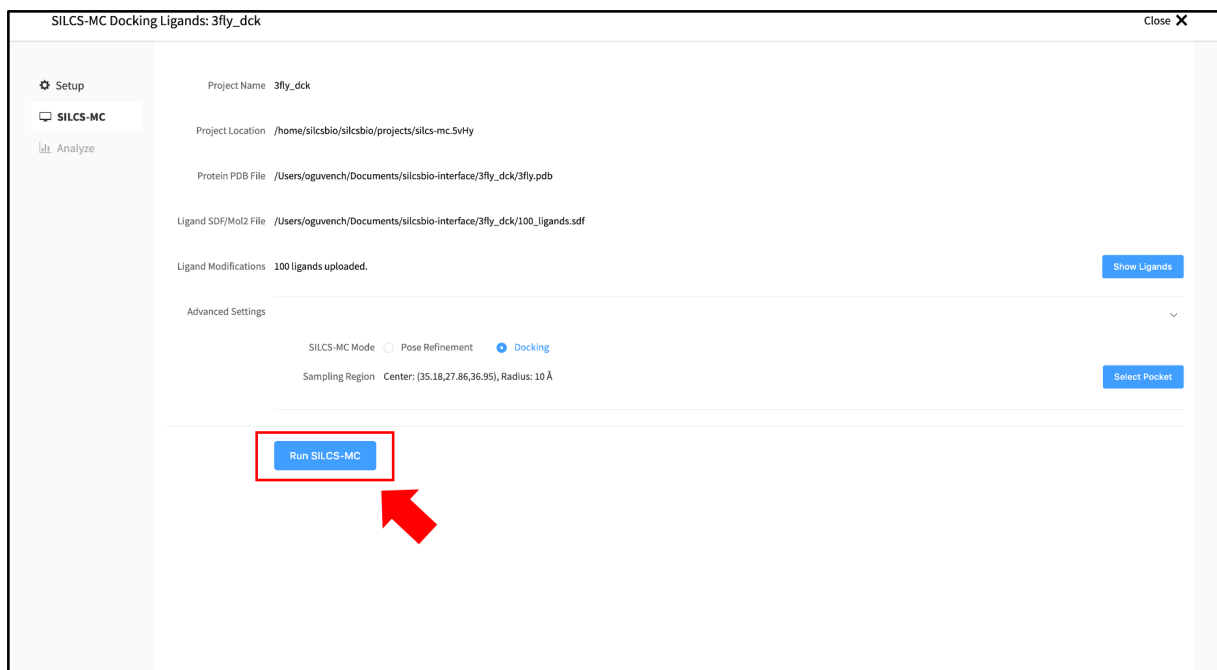
3. Once all information is entered correctly, press the “Setup” button at the bottom of the page. The page will update to list the number of ligands and show options for the sampling protocol (“Docking” or “Pose Refinement”) and the sampling region. Select the “Docking” option and then press the “Select Pocket” button.



4. The GUI will now be showing the protein molecular graphic in the center pane. On the right-hand side in the “Pocket” tab, you can define the pocket center based on the center-of-geometry of a ligand pose (“Define Pocket using Ligand”), or a target residue selection (“Define Pocket using Selection”), or by directly entering an x, y, z coordinate (“Define Pocket by XYZ”). You will also need to choose a radius (default value “10”) to complete the definition of the spherical pocket. If it is difficult to see the spherical pocket definition in the center pane, hide the protein surface representation. Click on the “Save Pocket” button and the “OK” acknowledgement to continue.



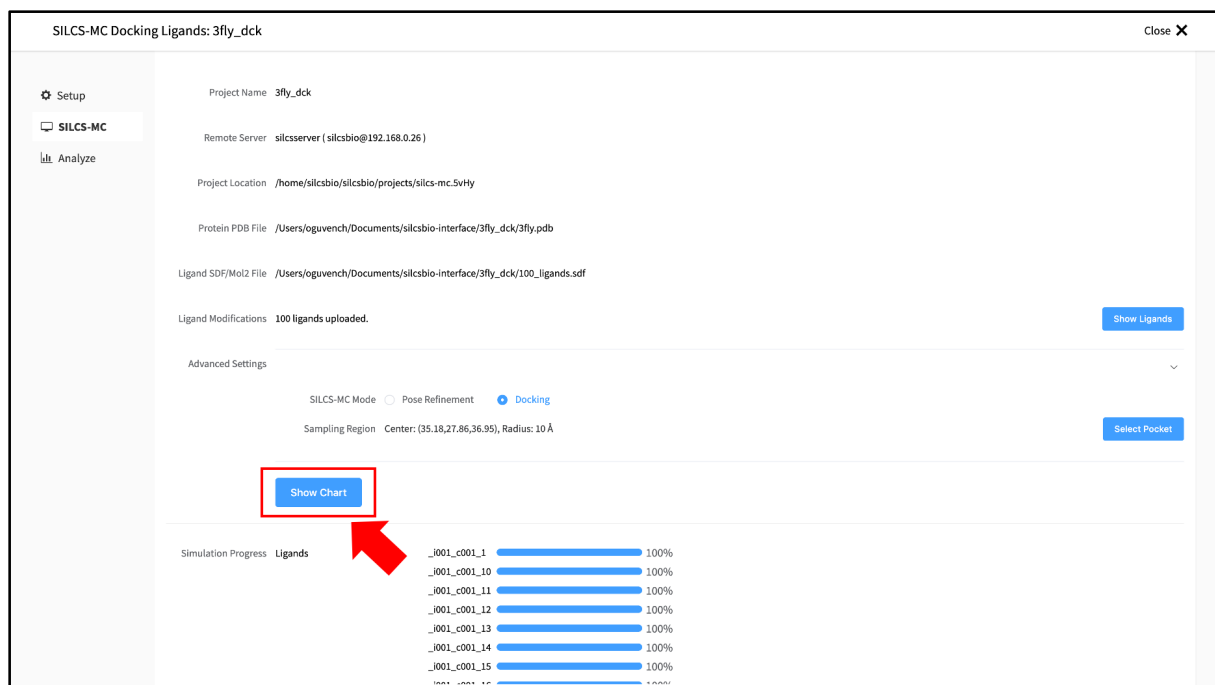
5. You will be returned to the previous screen, which now includes “Sampling Region” information consisting of the spherical pocket center and radius. You may now click the “Run SILCS-MC” button to start the SILCS-MC docking. Doing so will submit jobs to the remote server job scheduler and list them in a pop-over window.



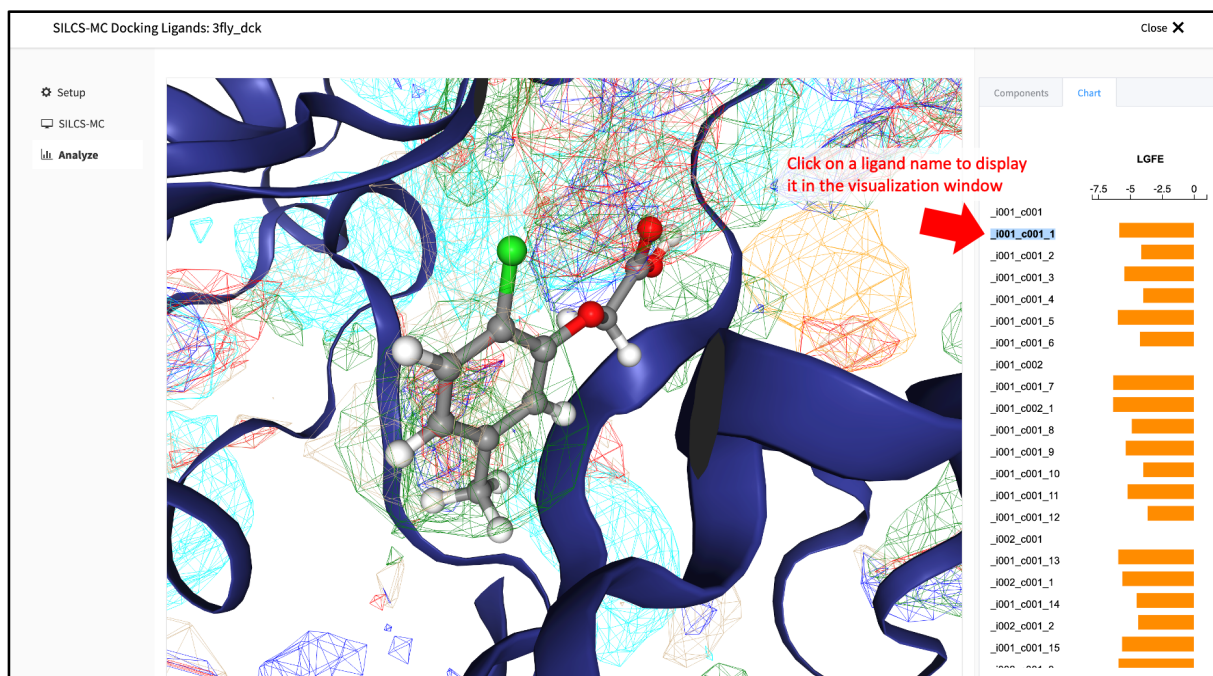
Once all jobs are submitted, you may click on the “Setup Successful” button to dismiss the

pop-over window and return to the previous screen, which will now show a “Simulation Progress” section. You may update this section by scrolling to the bottom of the screen and clicking the “Refresh” button. This will update the progress bars for all of the ligands being docked.

6. Once progress bars for all ligands reach 100%, click on the “Show Chart” button above the “Simulation Progress” section to proceed.



Upon successful completion of this command in the pop-over window, you may click on the green “Data collection finished” button to return to the GUI. A new tab, labeled “Chart” will have been created in the right-hand panel. Under that tab, clicking on a ligand name will center the visualization window at the ligand.



9.2.2 Running SILCS-MC docking from the command line interface

To set up and run SILCS-MC docking from the command line interface, create a directory containing all the ligands to be evaluated. Each ligand can be stored as a separate SDF or Mol2 file. Alternatively, all the ligands can be combined into a single SDF file.

```
${SILCSBIODIR}/silcs-mc/1_run_silcsmc_exhaustive \
  prot=<prot pdb> \
  ligdir=<directory containing ligand mol2/sdf> \
  mapsdir=<directory containing SILCS FragMaps> \
  center="x,y,z"
```

Note: .sdf, .sd, or .mol2 files can be placed in the `ligdir` directory, and SILCS-MC will read a single molecule from each file. Note that if a file contains multiple molecules, use of the `ligdir` option will result in only the first molecule in the file being processed.

If you have an SDF file with multiple molecules in it, replace `ligdir=<directory containing ligand mol2/sdf>` with `sdfile=<path to sdfile>` to process all molecules in the file.

9.2.3 SILCS-MC docking protocol details

Docking spawns five independent single-core serial jobs per ligand. Each run involves a maximum of 250 cycles and a minimum of 50 cycles of Monte Carlo/Simulated Annealing (MC/SA) sampling of the ligand within the defined 10 Å sphere. Each of these 250 cycles consists of 10,000 steps of MC at a high temperature followed by 40,000 steps of SA towards a lower temperature. At the beginning of each cycle, the ligand will be reoriented within the predefined sphere. When no sphere center is defined by the user, the ligand orientation in the input SDF or Mol2 file will be used as the starting pose for each cycle. The MC sampling has three types of moves: i) molecular translations with a maximum step size of 1 Å, ii) molecular rotation with a maximum step size of 180 degrees, and iii) intramolecular dihedral rotations with a maximum step size of 180 degrees. For intramolecular dihedral rotations, only the rotatable dihedral angles are selected for MC moves. The lowest LGFE scoring pose from the MC sampling is used as starting pose in the following SA sampling. The SA sampling also involves the same three types of moves, but with a smaller step size compared to the MC sampling: i) molecular translations with a maximum step size of 0.2 Å, ii) molecular rotation with a maximum step size of 9 degrees and, iii) intramolecular dihedral rotations with a maximum step size of 9 degrees. The lowest LGFE scoring pose from the SA is saved in a multi-frame PDB file: `3_silcsmc/<run>/pdb/<lig>_mc.<run>.pdb`.

In each run, after a minimum of 50 MC/SA cycles, if the LGFE score difference between the top three poses (defined by lowest LGFE scores) are less than 0.5 kcal/mol, then that run is considered converged and terminated. If the top three scored poses are separated by more 0.5 than kcal/mol, the MC/SA procedure continues either until the convergence criterion is met or until a maximum of 250 MC/SA cycles have been completed.

9.3 Running SILCS-MC pose refinement

The pose refinement protocol is designed to limit conformational sampling near the ligand input pose supplied by the user. Pose refinement is appropriate when there is high confidence in the input parent ligand pose and SILCS-MC evaluations are to be performed over a congeneric series. The sphere center for the pocket definition is the center-of-geometry from the input ligand pose.

9.3.1 Running SILCS-MC pose refinement from the SilcsBio GUI

Please see the previous section on running SILCS-MC docking from the SilcsBio GUI, and, in Step 3, select the “Pose Refinement” option. Note that there will be no “Select Pocket” step, as pose refinement assigns the pocket center based on the center-of-geometry of the input ligand. For multiple input ligands in a single `.sdf` file, each ligand will have its own center-of-geometry used to define the pocket center.

9.3.2 Running SILCS-MC pose refinement from the command line interface

To set up and run SILCS-MC pose refinement, create a directory containing all the ligands to be evaluated. Each ligand can be stored as a separate .mol2 or .sdf file. Alternatively, all the ligands can be combined into a single .sdf file.

```
${SILCSBIODIR}/silcs-mc/1_run_silcsmc_local prot=<prot pdb> \
  ligdir=<directory containing ligand mol2/sdf> \
  mapsdir=<directory containing SILCS FragMaps>
```

Note: .sdf, .sd, or .mol2 files can be placed in the `ligdir` directory, and SILCS-MC will read a single molecule from each file. Note that if a file contains multiple molecules, use of the `ligdir` option will result in only the first molecule in the file being processed.

If you have an SDF file with multiple molecules in it, replace `ligdir=<directory containing ligand mol2/sdf>` with `sdfile=<path to sdfile>` to process all molecules in the file.

9.3.3 SILCS-MC pose refinement protocol details

Pose refinement spawns five independent single-core serial jobs per ligand, and each run involves a maximum of 10 cycles of MC/SA sampling of the ligand within a 1 Å sphere. The center of the sphere is defined as the center-of-geometry of the input ligand pose. Each of cycles consists of 100 steps of MC at high temperature followed by 1000 steps of SA towards a lower temperature. At the beginning of each cycle, the ligand orientation/conformation will be reset to the one found in the input file. MC sampling moves are: i) molecular translation with a maximum step size of 1 Å, ii) molecular rotation with a maximum step size of 180 degrees, and iii) intramolecular dihedral rotation with a maximum step size of 180 degrees. For intramolecular dihedral rotation, only the rotatable dihedral angles are selected for MC moves. The lowest LGFE scoring pose from the MC sampling is used as starting pose in the following SA sampling. SA sampling moves are smaller than for the MC phase: i) molecular translation with a maximum step size of 0.2 Å, ii) molecular rotation with a maximum step size of 9 degrees, iii) intramolecular dihedral rotation with a maximum step size of 9 degrees. The lowest LGFE scoring pose from the SA is saved in the multi-frame PDB file `3_silcsmc/<run>/pdb/<lig>_mc.<run>.pdb`.

9.4 Best-pose retrieval

Once the SILCS-MC simulation is finished, retrieve the LGFE scores for each ligand subjected to SILCS-MC using:

```
${SILCSBIODIR}/silcs-mc/2_calc_lgfe_min_avg_sd ligdir=<directory_
↳containing lig mol2>
```

Note: If you used `sdfilename=<path to sdfilename>` option in the previous step, use `sdfilename=<path to sdfilename>` in this step as well, instead of `ligdir` option.

The above command will collect the SILCS-MC result and create `3_silcsmc/lgfe.csv` file, which contains the best LGFE scores from SILCS-MC sampling per ligand molecule. The command will collect top 10 best scoring poses and put them in `3_silcsmc/minconfpdb/` folder in SDF format.

Note: To change the number of poses, add `npose=<number>` at the end of `2_calc_lgfe_min_avg_sd` command.

To output PDB file format for best scoring poses, add `pdb=true` at the end of `2_calc_lgfe_min_avg_sd` command.

An example of the output of this script is:

LIG1	-34.587	-1.647
LIG2	-36.911	-1.605
LIG3	-36.131	-1.571
LIG4	-35.618	-1.619
LIG5	-36.586	-1.591
Name of Ligand	LGFE	LE

An alternative to the LGFE score is the ligand efficiency (LE). The LE is calculated as the LGFE score divided by the number of heavy atoms in each ligand.

$$LE = \frac{LGFE}{N_{\text{HeavyAtoms}}}$$

9.5 User-defined protocols

In addition to the default docking and pose refinement protocols, users can define their own SILCS-MC protocols. To do so, copy `${SILCSBIODIR}/templates/silcs-mc/params_custom.tmpl` to the location where you intend to run your custom SILCS-MC protocol. Edit this copy to reflect your customization; see below for a detailed description of user-

definable parameters. Parameter values in angle brackets in this file, such as <SILCSBIODIR>, will be replaced automatically at runtime where possible.

After you have edited `params_custom.tmpl`, use the following command to set up and run SILCS-MC. Each input ligand can be stored as a separate SDF or Mol2 file. Alternatively, all input ligands can be combined in a single SDF file.

```
${SILCSBIODIR}/silcs-mc/1_run_silcsmc_custom prot=<prot pdb> \
  ligdir=<directory containing ligand sdf/mol2> \
  mapsdir=<directory containing SILCS FragMaps> \
  paramsfile=<params_custom.tmpl>
```

The number of runs that will be spawned can also be modified with the command-line parameter `totruns`:

```
${SILCSBIODIR}/silcs-mc/1_run_silcsmc_custom prot=<prot pdb> \
  ligdir=<directory containing ligand sdf/mol2> \
  mapsdir=<directory containing SILCS FragMaps> \
  paramsfile=<params_custom.tmpl> \
  totruns=<# of runs>
```

The full list of user-definable parameters for `params_custom.tmpl` is:

- CGENFF_RULES <cgenff rules_file> (required)

This file is needed by the internal CGenFF library to determine the correct force-field parameters for the ligand. The default value is `${SILCSBIODIR}/data/cgenff.rules`

- CGENFF_PAR <cgenff parameter file> (required)

Along with the CGENFF_RULES file, this file is needed by the internal CGenFF library to determine the correct force-field parameters for the ligand. The default value is `${SILCSBIODIR}/data/par_all36_cgenff.prm`

- SILCS_RULES <silcs rules file> (required)

This rule file is used to map the different atoms in the ligand to the corresponding SILCS FragMap types. This mapping is used to determine the appropriate “field” that will be applied to the different atoms in the ligand when attempting an MC-move. The default value is `${SILCSBIODIR}/data/silcs_classification_rules_2018_generic_apolar_scale_1c.dat`

Another variant of this rule file is available at `${SILCSBIODIR}/data/silcs_classification_rules_2018_specific_standard_1c.dat`.

When `silcs_classification_rules_2018_generic_apolar_scale_1c.dat` is used, ligand atoms are assigned using six generic classifications in mapping them back to the FragMaps: APOLAR, HBDON, HBACC, MEOO, ACEO, and MAMN. All the atoms in the ligand fall into one of these six categories.

When `silcs_classification_rules_2018_specific_standard_1c.dat` is used, ligand atoms are assigned using 13 more specific classifications in mapping them back to FragMaps: BENC, PRPC, AALO, MEOO, FORN, FORO, IMIN, IMIH, ACEO, MAMN, APOLAR, HBDON and HBACC. For instance, aromatic ring carbons and aliphatic linker carbons are distinguished as BENC and PRPC, respectively here, while they are grouped as APOLAR atoms in the generic rule file.

- `GFE_CAP <default: 3.0>`

Maximum unfavorable GFE (kcal/mol) accounted in the MC calculation.

- `RDIE <default: true>`

When true, the distance dependent dielectric (RDIE) scheme is used to treat intramolecular electrostatics. When false, CDIE (constant dielectric scheme) is used.

- `DIELEC_CONST <default: 4>`

Dielectric constant used in the intramolecular electrostatic interactions calculations.

`#MINIMIZE_INPUT <default: false>`

Perform minimization of input structure.

`#MINIMIZE_BFGS <default: false>`

Perform minimization of input structure using BFGS algorithm.

- `MIN_STEPS <default: 10000>`

Maximum number of steps of minimization performed using the steepest-descent algorithm with the ligand, before initiating MC simulation.

- `EMTOL <default: 0.01>`

Minimization is converged when the diff in total energy (totE) across the last 10 steps is smaller than this value. Once this criteria is satisfied minimization terminates.

- `MC_MOVE_RANGE <default:1.0 180.0 180.0>.`

Maximum range of translation, rigid body rotation and dihedral rotation per step of MC simulation.

- `MC_PRNT_FRQ <default: 0>`

Number of intermediate steps of MC to be written into OUTMCPDBFILE.

- `MC_STEPS <default: 10000>`

Number of steps of MC simulation to be performed per cycle.

- `SIM_ANNEAL_MOVE_RANGE <default:0.2 9.0 9.0>`

Maximum range of translation, rigid body rotation and dihedral rotation per step of simulated annealing simulation after MC simulation.

- `SIM_ANNEAL_STEPS` <default: 40000>
Number of steps of simulated annealing to be performed per cycle.
- `INIT_RUNS` <default: 50>
Number of MC/SA cycles before initiating checks for convergence.
- `NUM_TOL` <default: 3>
Number of top-scoring cycles with differences in LGFE less than `DELTA_E_TOLERANCE`, before this simulation (run) is considered converged.
- `DELTA_E_TOLERANCE` <default: 0.5>
When differences in LGFE of `NUM_TOL` most-favorable cycles are less than this defined tolerance value, convergence is reached and the program exits
- `DELTA_E_BUFF` <default: 10>
Progression of MC+SA from one cycle to next is such that LGFE (of lowest conf) from MC should be less than (`prev_min+delta_e_buff`). This ensures that N cycles are proceeding towards a minimum lower than that previously discovered lowest energy conformation.
- `TOT_E_CRITERIA` <default: false>
When true, instead of LGFE, total energy (totE) of the system is used for convergence checks. Useful when running vacuum-phase MC simulations of the ligand.
- `TOT_RUNS` <default: 250>
Maximum number of MC simulation cycles. The program terminates if the `DELTA_E_TOLERANCE` criteria is satisfied before reaching `TOT_RUNS`. Alternately, even if the `DELTA_E_TOLERANCE` criteria is not satisfied when the number of cycles executed reaches `TOT_RUNS`, the program terminates.
- `RANDOM_SEED`: <default: system-time>
Seed used in MC simulation. When not set, system-time is used as a seed.
- `SIMULATION_CENTER`: <x, y, z>
Cartesian coordinates of where the MC simulation should be performed.
- `SIMULATION_RADIUS`: <default: 10.0 Å>
Radius of the sphere within which MC simulation will be performed.
- `RANDOM_INIT_ORIENT`: <true/false>
When set to TRUE, `SIMULATION_CENTER` should also be set. The ligand is placed within a sphere of size, `SIMULATION_RADIUS`, in a random orientation and a conformation
When set to FALSE, then the center-of-geometry of the ligand is used as the center for the MC simulation. This is useful when the ligand pose in the pocket is well known.

- `ATOM_TO_RESTRAIN`: <atom number in sdf/mol2>

When set, a spherical potential is applied to restrain the defined atom within the sphere during MC moves. This enables geometrically restraining a particular pharmacophore feature. Note, when using this feature, supply the full molecule with explicit hydrogens already added. Also, random pocket pose and placement using `RANDOM_INIT_ORIENT true` is incompatible.

When not set, the entire molecule is free to rotate/move/translate

- `ATOM_RESTRAINT_CENTER`: <x, y, z>

To be used in conjunction with `ATOM_TO_RESTRAIN` option. This value is used to defined the center of the spherical potential.

- `ATOM_RESTRAINT_RADIUS`: <default: 1.0 A>

To be used in conjunction with `ATOM_TO_RESTRAIN` option. This value is used to defined the radius of the spherical potential. When not defined, then a default of 1 A is used.

- `OUTRMSDFILE` <output RMSD file>

This file stores the RMSD and LGFE of the lowest energy conformation from each run of the MC/SA simulation. To be used in conjunction with `RANDOM_INIT_ORIENT` set to `true`.

- `SILCSMAP` <MapType> <map name> (required)

Multiple `SILCSMAP` flags can be defined, with each flag pointing to one file. Standard SILCS FragMaps of the following <MapType> should be included with the below keywords:

- EXCL - exclusion map
- NCLA - zero map for non-classified ligand atoms
- BENC - benzene carbon
- PRPC - propane carbon
- MEOO - methanol alcohol oxygen
- FORN - formamide nitrogen
- FORO - formamide oxygen
- MAMN - methyl ammonium nitrogen
- ACEO - acetate oxygens
- AALO - acetaldehyde oxygen
- IMINH - imidazole donor nitrogen
- IMIN - imidazole acceptor nitrogen
- APOLAR - generic non polar : green

- HBDON - generic donor: blue
- HBACC - generic acceptor: red
- OUTPUT_FORMAT [SDF|PDB] (required)
Choice of output format. SDF or PDB is supported.
- OUTPUT_FILE <output file name> (required)
This file stores the lowest energy conformation from each cycle of the MC/SA simulation.
- LOGFILE <output log file>
This file stores the energy statistics of the lowest energy conformation from each cycle of the MC/SA simulation.

SILCS-PHARM: RECEPTOR-BASED PHARMACOPHORE MODELS FROM FRAGMAPS

10.1 Background

Three-dimensional (3-D) pharmacophore models (also called “hypotheses”) offer an intuitive and powerful approach for virtual screening (VS). 3-D pharmacophore VS works by searching for matches between the 3-D pattern of functional groups constituting a pharmacophore model and the 3-D arrangement of functional groups in ligand conformers in a virtual database. Ligands having conformers that closely match the pharmacophore model are considered hits. Traditionally, 3-D pharmacophore models were developed using experimental knowledge of the binding poses for ligands to a receptor. This is in contrast to energy function-based VS (“docking”). An advantage of docking approaches was that they only require experimental knowledge of the receptor structure, and not of bound ligands. However, it is possible to develop pharmacophore models using only the receptor structure. One means to generate such receptor-based pharmacophore models is with data from SILCS simulations.

SILCS-Pharm converts Grid Free Energy (GFE) FragMaps into 3-D pharmacophore models. GFE FragMaps from SILCS simulations are used as inputs for the four-step SILCS-Pharm process:

1. voxel selection;
2. voxel clustering and FragMap feature generation;
3. FragMap feature to pharmacophore feature conversion;
4. generation of a pharmacophore model for virtual screening (VS).

Here, “feature” refers to the identity and location of a chemical functional group and “pharmacophore model” is a collection of pharmacophore features.

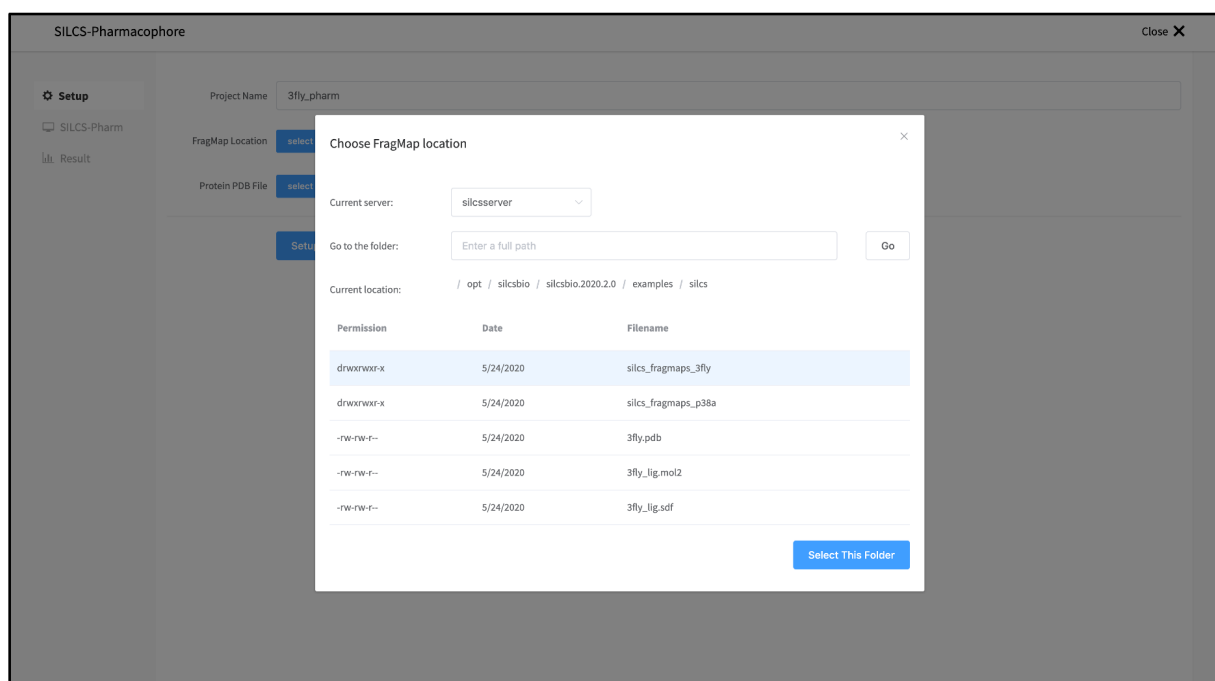
FragMap generation from SILCS MD entails partitioning 3-D space into uniform cubic voxels and enumerating fragment binding probabilities for each voxel. The voxels are retained during Boltzmann-inversion of probabilities to create GFE FragMaps. As the GFE voxel value represents the binding strength of a functional group at that specific location on the protein surface, the first step identifies the most favorable interactions based on a particular GFE cutoff value. In the second step, clustering is performed to group adjacent voxels, with each unique cluster becoming a

FragMap feature. In the third step, the FragMap features are classified and converted into pharmacophore features. Finally, the pharmacophore features are prioritized using Feature GFE (FGFE) scores to create a SILCS 3-D pharmacophore model [12]. In the current scheme, the program searches through five FragMap types: Generic Apolar, Generic Donor, Generic Acceptor, Methylammonium N, and Acetate O. Additionally, instead of using a rigidly-defined protein surface to determine regions that ligands cannot sample because of protein volume occlusion, the SILCS Exclusion Map is used. The Exclusion Map has been validated to be a better alternative to more traditional representations of the occluded volume of the protein, and it takes protein flexibility into account in an explicit way.

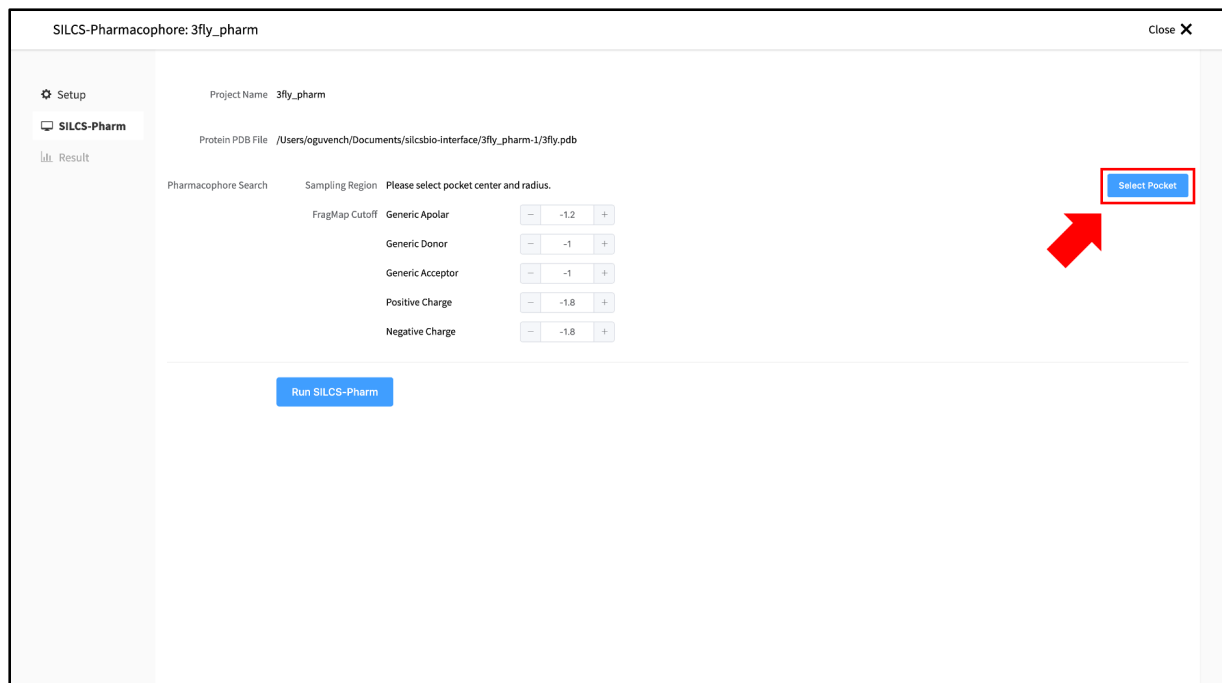
10.2 Running SILCS-Pharm

10.2.1 Running SILCS-Pharm from the SilcsBio GUI

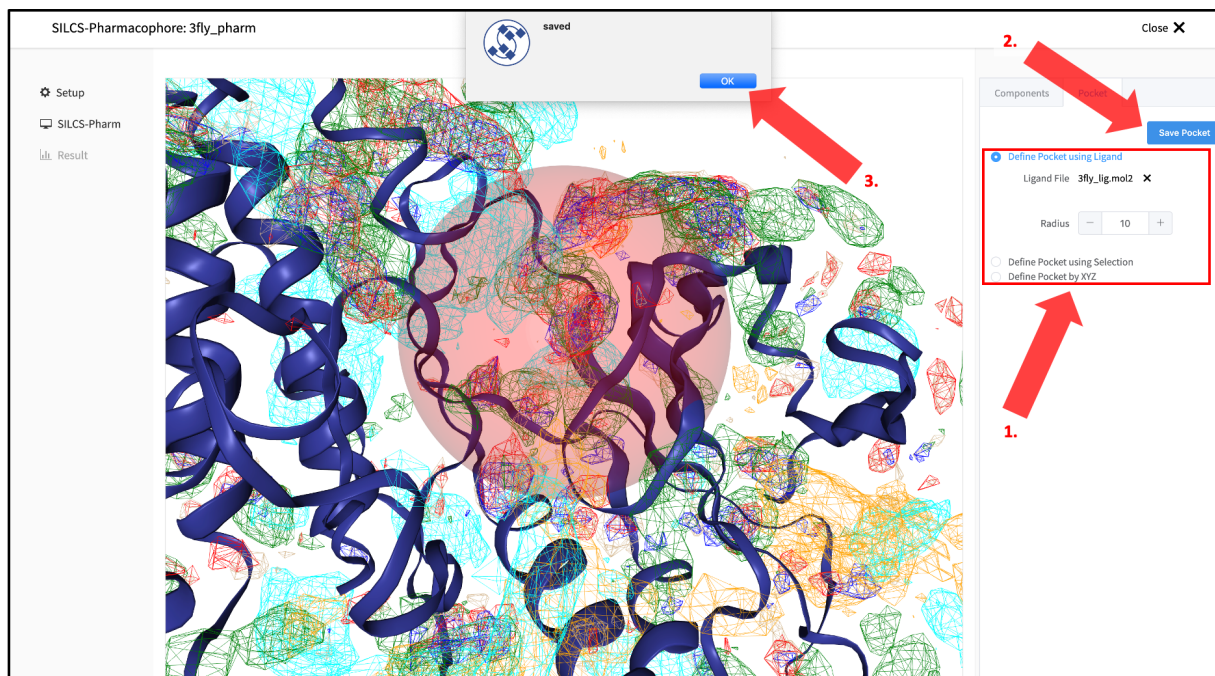
1. Select *New SILCS-Pharm project* from the Home page.
2. Enter a project name. Then, provide FragMap and protein input files. You may choose these files from the computer where you are running the SilcsBio GUI (“localhost”) or from any server you have previously configured, as described in [File and directory selection](#).



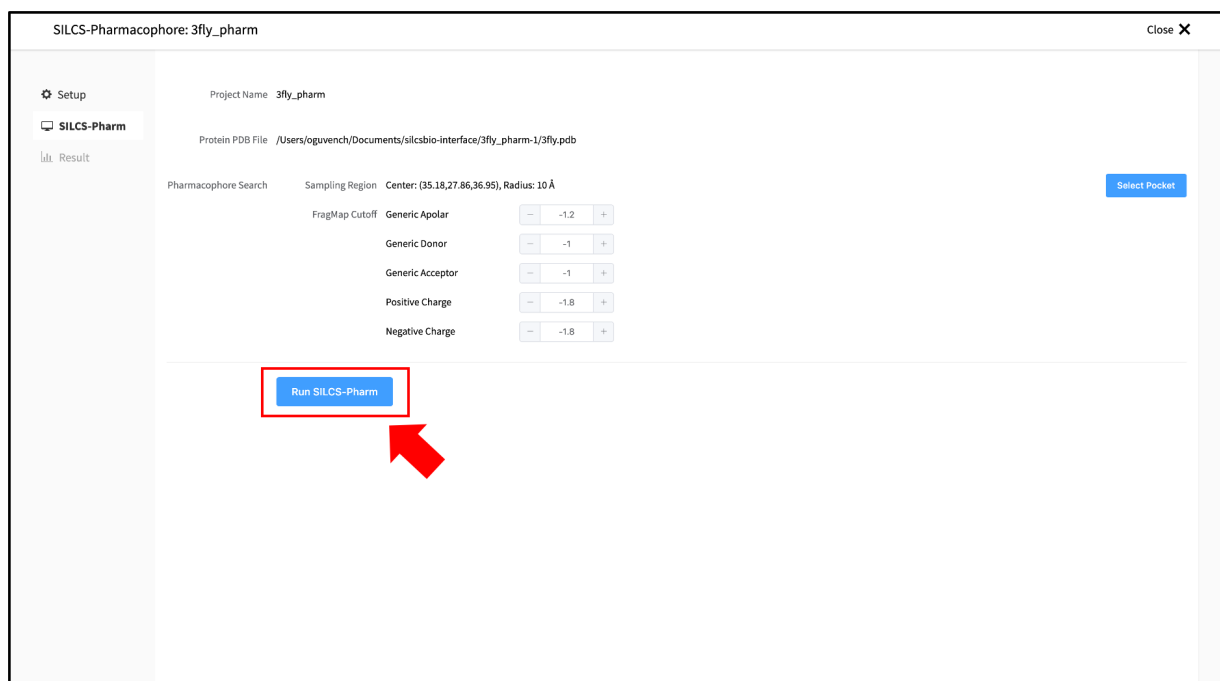
3. Once all information is entered correctly, press the “Setup” button at the bottom of the page. The screen will update to add “Pharmacophore Search” to the list of information. Click the “Select Pocket” button on the right-hand side of the window.



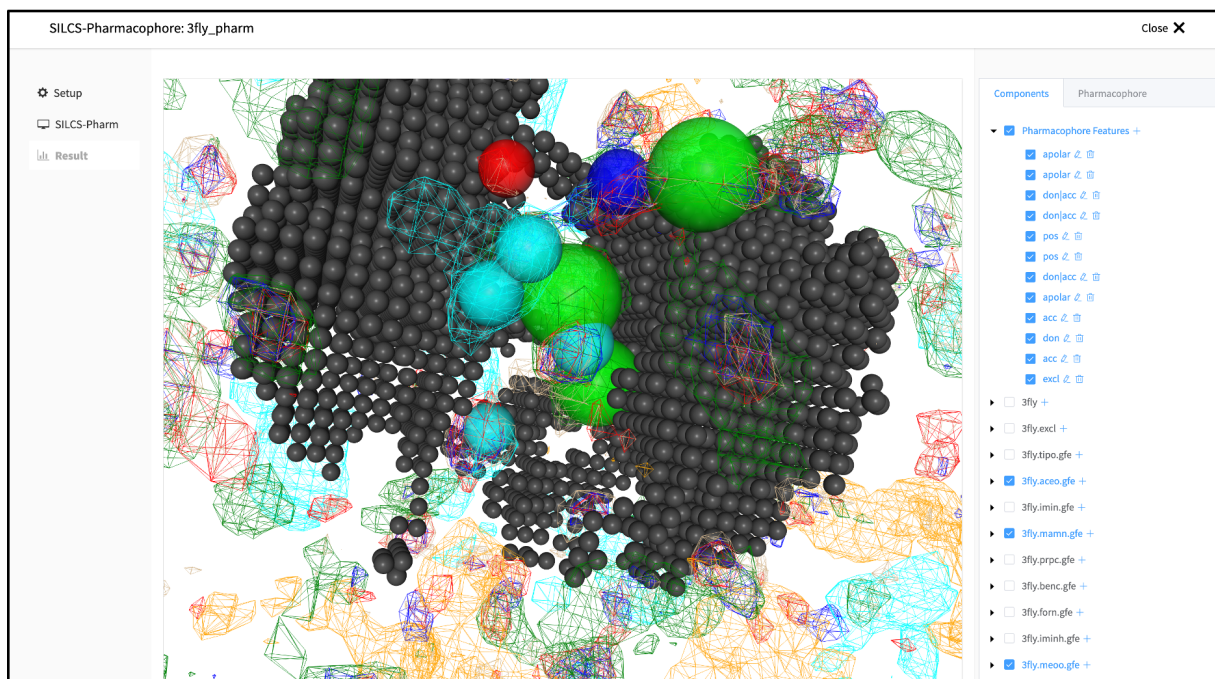
4. The GUI will now be showing the protein molecular graphic in the center pane. On the right-hand side, in the “Pocket” tab, you will need to define the pocket center based on the center-of-geometry of a ligand pose (“Define Pocket using Ligand”), or a target residue selection (“Define Pocket using Selection”), or by directly entering an x, y, z coordinate (“Define Pocket by XYZ”). You will also need to choose a radius (default value “10”) to complete the definition of the spherical pocket. If it is difficult to see the spherical pocket definition in the center pane, hide the protein surface representation. Click on the “Save Pocket” button and the “OK” acknowledgement to continue.



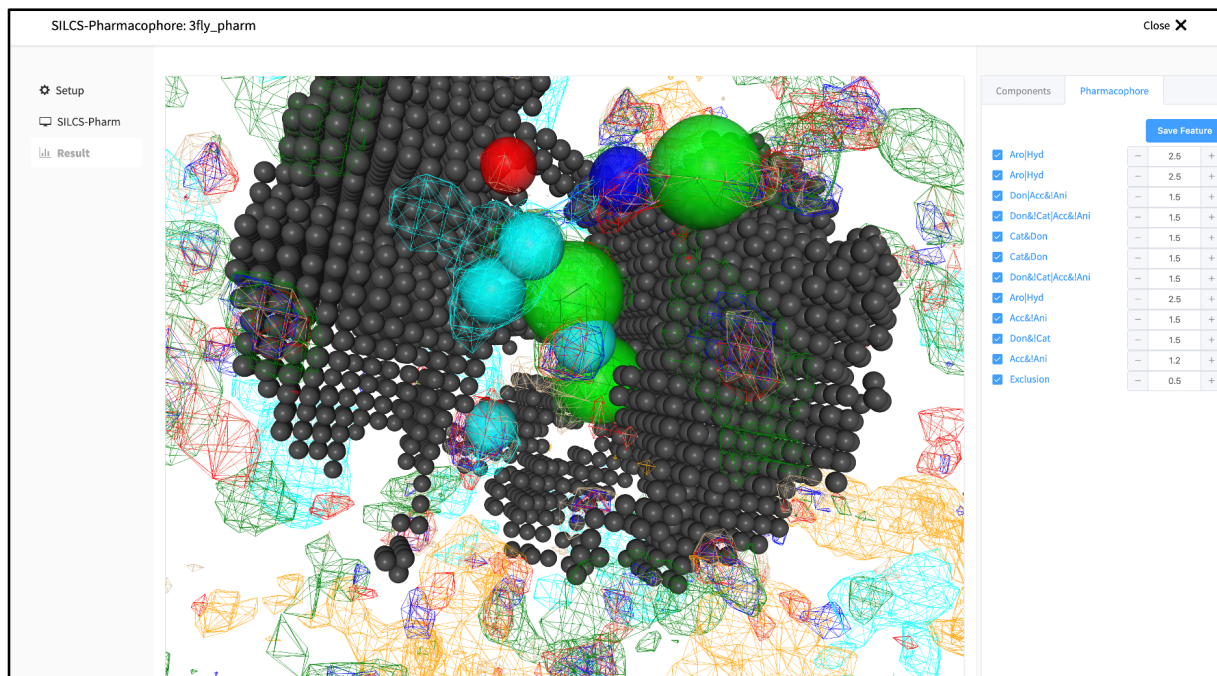
5. You will be returned to the previous screen, which now includes “Sampling Region” information consisting of the spherical pocket center and radius. You will also see default FragMap cutoff values listed for selection of FragMap densities for creation of pharmacophore features. You may adjust those values if you desire. Click on the “Run SILCS-Pharm” button to run the four-step SILCS-Pharm process.



A pop-over window will appear and show job output, and the job will run to completion in a matter of seconds. You may wish to click on the “Components” tab in the right-hand panel and deselect the protein for easier viewing.



The “Pharmacophore” tab will list all the pharmacophore features automatically generated from the FragMaps. You may adjust the radii of the individual features or deselect them entirely. Once finished with pharmacophore feature selection and adjustment, click the “Save Feature” button. This will allow you to save your pharmacophore model containing your selected/adjusted features in .ph4 format on your local computer. By selecting/adjusting different subsets of the pharmacophore features, including the Exclusion Map, you may create multiple different pharmacophore models and save each one as a separate .ph4 file. This capability allows for different pharmacophore model screens of a binding site. The resulting diverse hits from those screens can be combined and, for example, subjected to SILCS-MC Pose Refinement for rescoring.



10.2.2 Running SILCS-Pharm from the command line interface

A single command line interface command performs the four-step SILCS-Pharm process:

```
${SILCSBIODIR}/silcs-pharm/1_calc_silcs_pharm prot=<prot pdb> center=
  ↪ "x, y, z"
```

The input arguments are the PDB file used for the SILCS run and the absolute position of the center of a 10 Å sphere to be used to define the boundaries of the pharmacophore model. Two output files result from this command. <prot>.keyf_<#features>.ph4 can be directly used for 3-D pharmacophore VS by compatible programs (see below for generating Pharmer-compatible ph4 files). <prot>_silcspharm_features.pdb provides output in PDB format for easy visualization using standard molecular graphics packages. Running the command with no arguments

```
${SILCSBIODIR}/silcs-pharm/1_calc_silcs_pharm
```

will list additional options. Along with the required arguments of prot=<prot pdb> and center="x, y, z", the following additional options can be set:

1) FragMap directory path:

```
mapsdir=<location and name of directory containing FragMaps; ↵
  ↪ default=2b_gen_maps>
```

By default, the program looks for FragMaps in the 2b_gen_maps directory.

2) Output directory path:

```
outputdir=<location and name of output directory; default=5_pharm>
```

By default, the program creates the directory 5_pharm and places all output files there.

3) Radius:

```
radius=<default: 10>
```

By default, a radius of 10 Å centered at center="x,y,z" is searched to generate pharmacophore features from the input FragMaps.

4) Generic Apolar FragMap cutoff:

```
apolar_cutoff=<default: -1.2>
```

By default, Generic Apolar FragMap voxels having a GFE value ≤ -1.2 kcal/mol are selected.

5) Generic Donor FragMap cutoff:

```
hbdon_cutoff=<default: -1.0>
```

By default, Generic Donor FragMap voxels having a GFE value ≤ -1.0 kcal/mol are selected.

6) Generic Acceptor FragMap cutoff:

```
hbacc_cutoff=<default: -1.0>
```

By default, Generic Acceptor FragMap voxels having a GFE value ≤ -1.0 kcal/mol are selected.

7) Methylammonium N FragMap cutoff:

```
mamn_cutoff=<default: -1.8>
```

By default, Methylammonium N FragMap voxels having a GFE value ≤ -1.8 kcal/mol are selected.

8) Acetate O FragMap cutoff :

```
aceo_cutoff=<default: -1.8>
```

By default, Acetate O FragMap voxels having a GFE value ≤ -1.8 kcal/mol are selected.

In addition to visualization, the output PDB file `<prot>_silcspharm_features.pdb` can be used for easy editing of the pharmacophore model. Using a text editor, modify/reduce the features in this file as desired and save the revised file as

<prot>_silcspharm_features_revise.pdb. Using this revised PDB file and the original ph4 file <prot>.keyf_<#features>.ph4 as input, create a new ph4 file with:

```
${SILCSBIODIR}/programs/revise_ph4 <prot>.keyf_<#features>.ph4
➔<prot>_silcspharm_features_revise.pdb
```

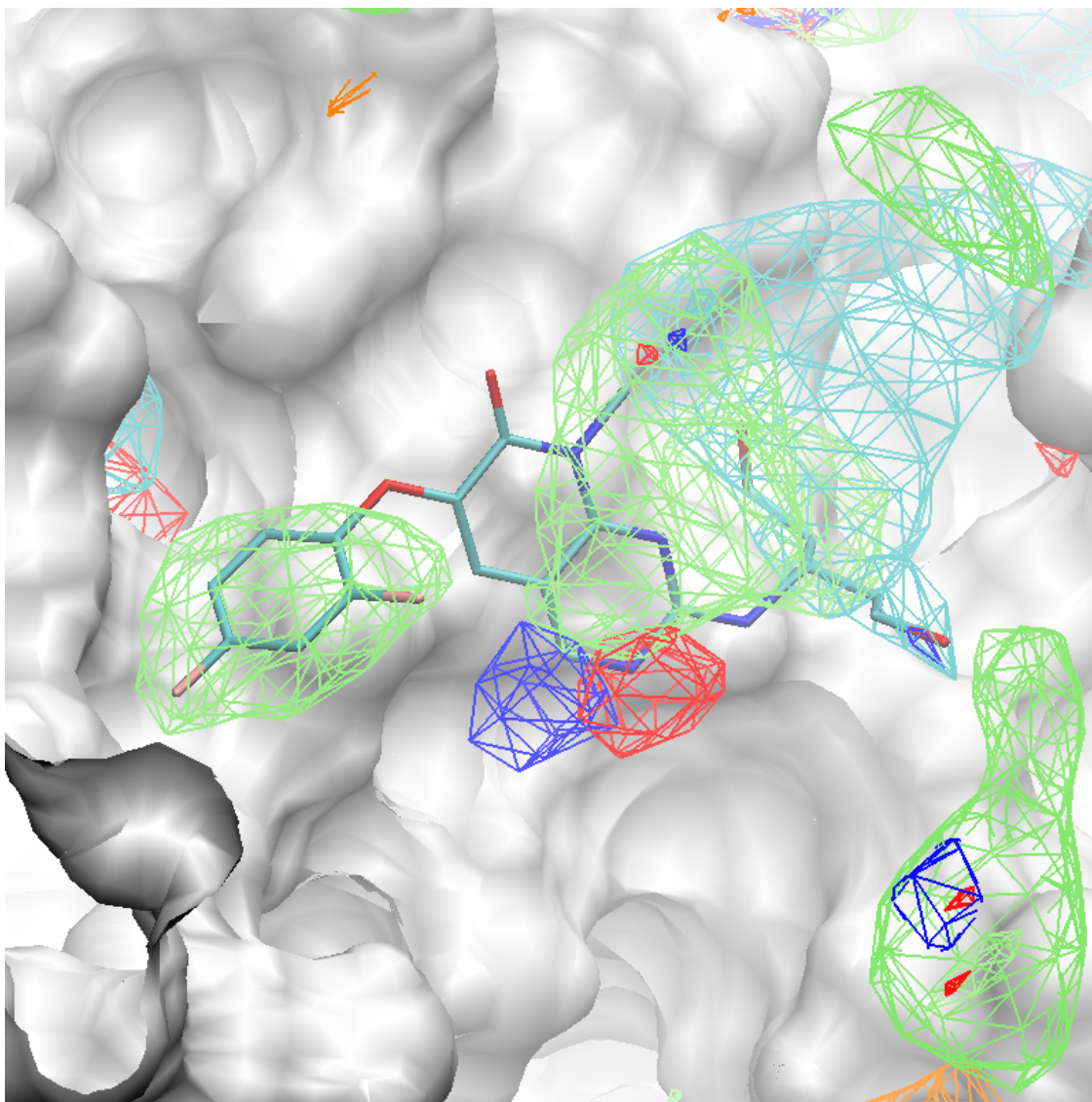
The output of this command will be the revised ph4 file <prot>.keyf_<#features>_revise.ph4. To create Pharmer-compatible ph4 output, add the pharmer option:

```
${SILCSBIODIR}/programs/revise_ph4 <prot>.keyf_<#features>.ph4
➔<prot>_silcspharm_features_revise.pdb pharmer
```

10.2.3 Example

The following example demonstrates use of SILCS-Pharm from the command line interface to generate a pharmacophore model for p38 MAP kinase. Input files, including FragMaps, are provided in `${SILCSBIODIR}/examples/silcs/` (these same input files may also be used for a trial run of SILCS-Pharm with the SilcsBio GUI).

The x,y,z coordinates of the center of the complexed ligand are used to create a SILCS-Pharm 3-D pharmacophore model encompassing the ligand binding site (shown below). These coordinates define the center of the sphere within which FragMap voxels are searched and clustered to generate features.



Using the ligand center coordinates `center="35.24, 27.48, 37.73"`, generate the 3-D pharmacophore model with:

```
${SILCSBIODIR}/silcs-pharm/1_calc_silcs_pharm prot=3fly.pdb center="35.
→24,27.48,37.73" mapsdir=${SILCSBIODIR}/examples/silcs/silcs_fragmaps_
→3fly/maps
```

The command will complete after several seconds, and the output will note, “A total of 13 features have been detected.” All output files will be in a new subdirectory `5_pharm`. Standard molecular graphics software can be used to visualize the output file `3fly_silcspharm_features.pdb`, which has one `ATOM` entry for each of the 13 pharmacophore features:

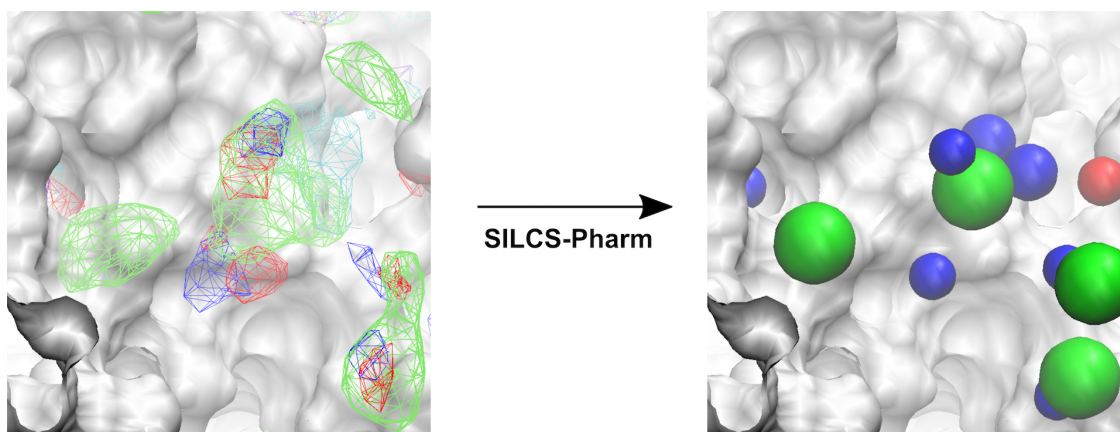


Fig. 10.1: Conversion of FragMaps (left) to SILCS pharmacophore features (right).

To modify/reduce the features, edit the output file `3fly_silcspharm_features.pdb` and save it as `3fly_silcspharm_features_revise.pdb`, then run `${SILCSBIODIR}/programs/revise_ph4`:

```
${SILCSBIODIR}/programs/revise_ph4 3fly.keyf_13.ph4 3fly_silcspharm_
→features_revise.pdb
```

The output `3fly.keyf_13_revise.ph4` will reflect your revisions in `3fly_silcspharm_features_revise.pdb`.

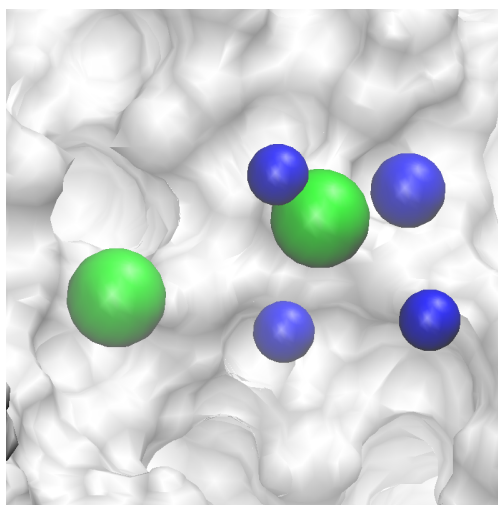


Fig. 10.2: Revised SILCS pharmacophore features.

SILCS-HOTSPOTS: FRAGMENT BINDING SITES INCLUDING ALLOSTERIC SITES

11.1 Background

SILCS-Hotspots identifies all potential fragment binding sites on a target by doing SILCS-MC sampling across the entire target structure. The protein or other macromolecular target is partitioned into a collection of subspaces in which the fragment is randomly positioned and subjected to extensive SILCS-MC to identify favored local poses. This may be performed 1000 times or more per fragment in each subspace. The identified fragment positions are then RMSD-clustered followed by selection of the lowest energy pose in each cluster to define a binding site for further analysis.

SILCS-Hotspots may be applied to small fragment-like molecules as well as larger drug-like molecules. When multiple fragments are used, a second round of clustering may be performed over the different fragment types to identify binding sites occupied by one or more of the fragment types included in the calculation.

Results from SILCS-Hotspots include all potential binding poses of the individual fragments, binding sites that contain one or more of the fragment types, and ranking of fragment poses and binding sites based on LGFE scores. Applications of SILCS-Hotspots include identifying putative fragment binding sites and performing fragment-based drug design. Putative binding sites identified by SILCS-Hotspots can be used for rapid database screening with SILCS-Pharmacophore. Alternatively, sites identified by SILCS-Hotspots can be considered for fragment-based design by linking poses of fragment-like molecules in adjacent sites to create drug-like molecules.

11.2 Usage and Options

11.2.1 Running SILCS-Hotspots calculations

Inputs for SILCS-Hotspots are the protein or other macromolecular target used for the SILCS simulations, the SILCS FragMaps resulting from those simulations, and the fragment(s) to be

used for sampling. SilcsBio provides fragment files appropriate for SILCS-Hotspots (see below). Alternatively, you may use your own fragment file(s).

```
$SILCSBIODIR/silcs-hotspots/1_setup_silcs_hotspots prot=<prot PDB>
↳ligdir=<fragment database> mapsdir=<mapsdir> bundle=<true/false>
```

The input arguments are the PDB file used for the SILCS run (`prot`), and the locations of the fragment files (`ligdir`) and SILCS FragMaps (`mapsdir`). When SILCS-Hotspots calculations are performed, the subdirectory `4_hotspots/` is created to store output from the exhaustive SILCS-MC runs.

Note: The above command will submit a large number of jobs, which can strain job queueing systems. The `bundle` keyword launches bundled jobs instead of individual jobs.

Options include:

1) FragMap directory path:

```
mapsdir=<location and name of directory containing FragMaps;
↳default=maps>
```

By default, the program looks for FragMaps in the `maps/` directory.

2) Fragment database location:

```
ligdir=<location and name of directory containing fragment mol2/
↳sdf>
```

You may prepare your own directory with Mol2/SDF files. There are also three directories of fragment-like molecules provided with your SilcsBio software:

- `$SILCSBIODIR/data/databases/ring_system`: Mono- and bicyclic rings commonly appearing in drug-like compounds [7].
- `$SILCSBIODIR/data/databases/ring_subset`: A small subset of the `ring_system` database.
- `$SILCSBIODIR/data/databases/astex_mini_frag`: Polar fragments curated from Astex [8].

3) Number of processors to use for bundled jobs:

```
nproc=<# of processors used when bundle=true>
```

4) Directory containing output to be used in subsequent steps:

```
hotspotsdir=<location of hotspots data; default=4_hotspots>
```

5) Template file for SILCS-MC sampling:

```
paramsfile=<custom params file>
```

The default SILCS-MC job parameters file for SILCS-Hotspots is `$SILCSBIODIR/templates/silcs-hotspots/params.tmpl`. See `mcilcs` for customization details.

11.2.2 Post-run clustering

After all the SILCS-Hotspots jobs are complete, the next step is to cluster fragment poses that were output by the completed jobs. The clustering algorithm iteratively finds clusters with the largest number of members [9]. The process entails 1) computing the number of neighbors of each pose, 2) choosing the pose with the largest number of neighbors and marking it and its neighbors as cluster members, 3) removing cluster members identified in Step 2 from the pool of poses, and 4) repeating Steps 1-3 until there are no available poses left. The cluster “center” is defined as that fragment pose with the most neighbors in that cluster. This pose’s LGFE score defines the LGFE score of the cluster. Run the command

```
$SILCSBIODIR/silcs-hotspots/2_collect_hotspots prot=<prot PDB> ligdir=
↳<fragment database>
```

to perform the clustering. You may also specify the following additional options:

1) Radius for RMSD clustering:

```
cutoff=<cutoff for clustering; default=3>
```

2) Maximum number of sites to be identified for each fragment:

```
maxsites=<maximum number of sites ordered by LGFE; default=200>>
```

3) Directory containing output from the previous steps:

```
hotspotsdir=<location of hotspots data; default=4_hotspots>
```

Note: `2_collect_hotspots` and `3_create_report` have external Python dependencies. You may need to install the following Python packages (for example by using `pip install <package name>`):

- numpy
- scipy
- tqdm
- pyyaml

- `xlsxwriter`

11.2.3 Site determination and report generation

The final step of SILCS-Hotspots is site determination and report generation. This involves a second round of clustering over all specified fragments to identify sites on the protein to which one or more fragments bind. Each site is given the average LGFE score over all the fragments. In addition, the sites themselves may be clustered to identify binding pockets that suggest multiple adjacent sites that may be linked to build larger fragments (under development). Information on all the fragments, the binding sites, and the binding pockets is included in `report.xlsx` and in PDB files that are output to the subdirectory `4_hotspots/report/`. Run the following command

```
$SILCSBIODIR/silcs-hotspots/3_create_report ligdir=<fragment database>
```

You may add these options:

- 1) Directory containing output from the previous steps:

```
hotspotsdir=<location of hotspots data; default=4_hotspots>
```

- 2) Cluster radius for binding site determination:

```
site_cutoff=<cluster radius for site determination; default=6.0>
```

- 3) LGFE cutoff for inclusion of fragment poses in site determination:

```
ligand_lgfe_cutoff=<Ligand LGFE cutoff for site determination;   
↪default=-2.0>
```

- 4) Average LGFE cutoff for site determination:

```
site_lgfe_cutoff=<average LGFE cutoff for binding sites; default=  
↪-2.0>
```

Sites having values less favorable than the cutoff will be discarded.

- 5) Flag to activate binding pocket calculation and associated clustering radius (under development):

```
pocket=<perform pocket analysis; default=False>  
pocket_cutoff=<cluster radius for binding pocket determination;   
↪default=12.0>
```

The `3_create_report` command will populate the `4_hotspots/report/` subdirectory with the following:

- `report_all.xlsx`: Spreadsheet file containing analysis information. Included are the LGFE and Ligand Efficiency (LE) scores for each copy of every fragment obtained from clustering, relative affinity analysis, and listing of the posed fragment that defines each site. If binding pocket analysis is performed, information on binding pockets is included.
- `hotspots_sites.pdb`: PDB file containing the identified sites. The B-factor column value includes the average LGFE score of that site.
- `pdb_by_ligands`: Directory containing PDB files for each fragment with multiple coordinates for each site identified for the fragment. In the PDB files, REMARK includes the LGFE scores, the B-factor column includes the GFE score for each atom, and the final column lists the SILCS atom type.
- `pdb_by_site`: Directory containing PDB files of the fragments located at each site in `hotspots_sites.pdb`. For example, the filename `site_all_1_8_1.pdb` indicates that at site 1 fragment 8 is present. The final 1 indicates that this is the first copy of fragment 8 at site 1. From the clustering algorithm, it is possible that more than one copy of a fragment is included in a site. For example, `site_all_1_8_2.pdb` would be the second copy of fragment 8. In each PDB file, REMARK includes the LGFE scores, the B-factor column includes the GFE score for each atom, and the final column lists the SILCS atom type.

The following additional outputs are produced if binding pocket analysis is performed (`pocket=true`):

- `hotspots_pockets.pdb`: PDB file containing sites that define each binding pocket. In the following, pocket P01 or 1 is defined by four sites with LGFE values for each site shown in the B-factor column. The algorithm does not number pockets consecutively: in this example, there is no P02 pocket, and pocket P03 contains 2 sites.

ATOM	1	X	P01	A	1	20.980	7.230	-12.513	1.00	-4.39	␣
↪		C									
ATOM	2	X	P01	A	1	27.947	16.848	-6.986	1.00	-2.82	␣
↪		C									
ATOM	3	X	P01	A	1	17.606	12.224	-10.043	1.00	-2.67	␣
↪		C									
ATOM	4	X	P01	A	1	14.912	17.557	-5.282	1.00	-2.08	␣
↪		C									
ATOM	5	X	P03	A	3	16.182	-10.455	11.793	1.00	-2.96	␣
↪		C									
ATOM	6	X	P03	A	3	17.605	0.058	15.384	1.00	-2.83	␣
↪		C									

- `pdb_by_pocket`: Directory containing PDB files for fragments that comprise each pocket. For example, `pocket_all_8_site_12_10_1.pdb` indicates pocket 8 contains a fragment from site 12 and that fragment is fragment 10. 1 indicates that it is the first copy of fragment 10 in that pocket. REMARK includes the LGFE scores, the B-factor column includes the GFE score for each atom, and the final column is the SILCS atom type.

11.3 Practical Considerations

`1_setup_silcs_hotspots` creates a subdirectory, `4_hotspots/`, (or user defined name using `hotspotsdir=`) that contains fragment pose and LGFE information. As SILCS-Hotspots makes use of large numbers of SILCS-MC calculations on each fragment, this directory will be filled with a substantial amount of data. It is suggested that, once all analyses are complete, these files either be deleted or archived, and `4_hotspots/` be renamed prior to additional hotspots runs. Remember, the data in `4_hotspots/` are used for post-run clustering and site determination and report generation.

If `1_setup_silcs_hotspots` is being rerun with new parameters, such as new fragments as specified by `ligdir=`, the subdirectory `4_hotspots/` should be renamed or an alternate name assigned using `hotspotsdir=` to avoid information from the original run being overwritten. However, this is not strictly necessary IF all the new fragments have unique filenames relative to the original run AND the SILCS-MC job parameters specified by `paramsfile=` are not changed, since the subsequent `2_collect_hotspots` command only performs analysis on Mol2/SDF files in the specified `ligdir` directory.

`1_setup_silcs_hotspots` launches a large number of jobs that, while the majority of jobs finish quickly individual jobs may take time (minutes to an hour) to complete. In some cases one or two jobs in the set may require additional time due to the robust SILCS-MC convergence criteria used in SILCS-Hotspots.

For the SILCS-MC sampling, especially with larger fragments or ligands, you may prefer not to include the SILCS exclusion map. This will permit fragment sampling of poses that would otherwise be rejected because of overlap with the exclusion region. Using this approach, final fragment poses will be based solely on scoring with SILCS FragMaps. To achieve this, set the weighting of the exclusion map to zero in your custom SILCS-MC job parameters file (`paramsfile=<custom params file>`). A simple means to this end is to use a copy of the default file `$SILCSBIODIR/silcs-mc/params_custom.tmpl` in which you replace the `1.000` in the below line with `0.000`.

<code>SILCSMAP EXCL <MAPDIR>/<prot>.excl.map</code>	<code>1.000</code>
---	--------------------

Clustering of data in the `4_hotspots/` or equivalent subdirectory with the `2_collect_hotspots` command produces representative fragment poses on a per-cluster basis. If clustering is repeated, for example with a different clustering radius, the old PDB files created in `4_hotspots/` will be overwritten. Therefore, make sure to run site determination and report generation (the `3_create_report` command) on your original clustering output before repeating clustering.

`2_collect_hotspots` processes each of the fragments individually and typically requires minutes to complete for 100 fragments. During that process a number of warning messages, such as “Clustered PDB not found: `4_hotspots/2/subspace_1/pdb/2_clust_1_1.pdb`,” will be given. These are expected as they indicate subspaces for the fragments in which no favorable fragments poses were identified. For example, this may occur where the subspace encompasses the protein

structure.

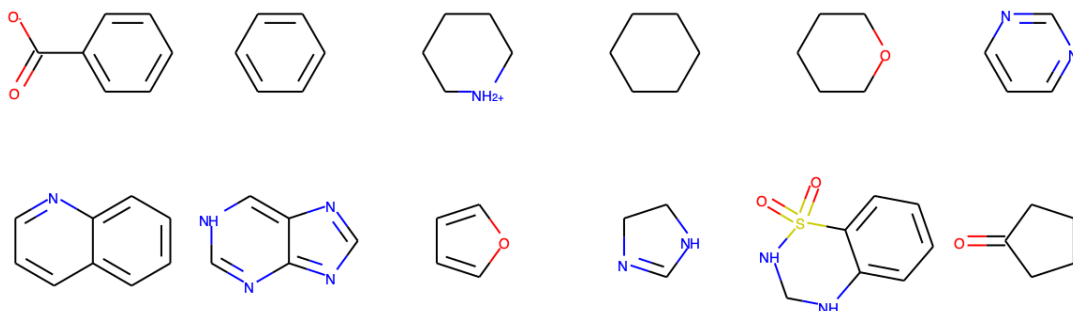
Site determination and report generation with the `3_create_report` command creates a subdirectory, `4_hotspots/report/`, and outputs `report.xls` and PDB files into this subdirectory. Rerunning `3_create_report` with different parameters, like an alternate value of the site clustering cutoff, will overwrite the original output. Therefore, prior to rerunning report generation, rename `4_hotspots/report/` to save your original report generation outputs. The clustering algorithm for site determination does not consider the identity of the fragment when performing the clustering. Accordingly, in certain cases it is possible for the same fragment to be included two or more times in a given site.

11.4 Example

The following demonstrates use of SILCS-Hotspots on p38 MAP kinase. Input files, including FragMaps, are in `$SILCSBIODIR/examples/silcs/`.

```
cp -r $SILCSBIODIR/examples/silcs/silcs_fragmaps_p38a .
cd silcs_fragmaps_p38a
$SILCSBIODIR/silcs-hotspots/1_setup_silcs_hotspots prot=p38a.pdb_
  ↳ligdir=$SILCSBIODIR/data/databases/ring_subset mapsdir=maps_
  ↳bundle=true
```

In this example, we use `$SILCSBIODIR/data/databases/ring_subset` as the fragment database. Owing to the small size of this database, the example run finishes quickly. Below are the ring fragments in the `ring_subset` database.

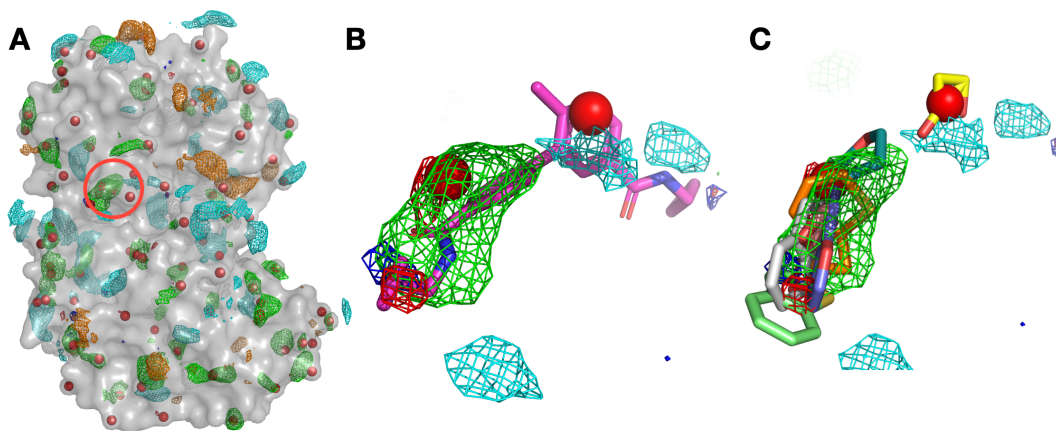


Once the jobs spawned by `1_setup_silcs_hotspots` complete, use the following commands for post-run clustering and site determination and report generation.

```
$SILCSBIODIR/silcs-hotspots/2_collect_hotspots prot=p38a.pdb ligdir=
  ↳$SILCSBIODIR/data/databases/ring_subset
$SILCSBIODIR/silcs-hotspots/3_create_report ligdir=$SILCSBIODIR/data/
  ↳databases/ring_subset
```

This will have created the `4_hotspots/report/` subdirectory containing the following:

- `hotspots_sites.pdb`: Centroid positions of fragment clusters, that is, the “hotspots.” Clusters are ranked using average LGFE scores, with the cluster rank listed in the residue number field and the average LGFE score in the B-factor field.
- `report_all.xlsx`: Report of hotspots in spreadsheet format.
- `pdb_by_sites`: List of PDB files for each hotspot.



Panel (A) shows p38 (surface representation), SILCS FragMaps (wire frame), and all hotspots (red spheres) determined by SILCS-Hotspots. The crystal binding pocket is circled in red. Panel (B) has FragMaps, hotspots, and the crystallographic ligand. Two hotspots are identified within the crystal binding pocket and coincide with the rings of the fragment. Panel (C) shows fragment poses from SILCS-Hotspots calculations as well as FragMaps and hotspots.

11.5 Excipient design for biologics

Please contact info@silcsbio.com for more information on this capability.

SSFEP: SINGLE STEP FREE ENERGY PERTURBATION

12.1 Background

Free energy perturbation (FEP) has long been considered the gold standard in calculating relative ligand-binding free energies. However, FEP is often impractical for evaluating large number of changes to a parent ligand due to the large computational cost. Single Step Free Energy Perturbation (SSFEP) is an alternative that can be orders of magnitude faster than conventional FEP when evaluating large number of changes to a parent ligand, while maintaining useful accuracy for small functional group modifications [13].

The SSFEP method involves post-processing of MD simulation data of a ligand in a given environment in the canonical ensemble to estimate the alchemical free energy change of chemically modifying the ligand. Zwanzig's FEP formula is used,

$$\Delta G_{L1 \rightarrow L2}^{\text{env}} = -k_B T \ln \langle e^{-\beta \Delta E} \rangle_{L1} \quad (12.1)$$

where k_B is the Boltzmann constant and T is the temperature. The angular brackets indicate an average of the exponential factor over the MD trajectory of ligand $L1$ in the given environment, env , which can be either the solvated protein or water. ΔE is the energy difference between the two systems involving $L1$ and $L2$, which in practice is computed as the difference in the interaction energies of the two ligands in the corresponding environment:

$$\Delta E = E_{L2-\text{env}} - E_{L1-\text{env}}$$

The environment env in each system is defined as all non-ligand atoms. As the environment is constant between the two ligands, the internal environmental energy cancels exactly during the computation of ΔE . In addition, as the difference between $L1$ and $L2$ involves a very small number of heavy atom modifications, we expect any differential intra-ligand energy terms to also cancel exactly between the solution and protein environments. Therefore, once $\Delta G_{L1 \rightarrow L2}^{\text{protein}}$ and $\Delta G_{L1 \rightarrow L2}^{\text{water}}$ are computed according to Eq. (12.1), the relative binding free energy is given by

$$\Delta \Delta G_{L1 \rightarrow L2}^{\text{bind}} = G_{L1 \rightarrow L2}^{\text{protein}} - G_{L1 \rightarrow L2}^{\text{water}}$$

The SSFEP approach allows the data from simulation of a single protein-ligand complex to be rapidly post-processed to evaluate tens to hundreds of potential modifications involving multiple

sites on the parent ligand. Given this, the best results are achieved when SSFEP is used to evaluate small modifications to the parent ligand.

In a recent study [14], the ability of standard FEP and SSFEP to reproduce the experimental relative binding affinities of known ligands for two proteins, ACK1 and p38 MAP kinase, was tested. SSFEP was able to produce comparable results to full FEP while requiring a small fraction of the computational resources.

12.2 Usage

The following usage details are provided for completeness. **We strongly recommend using the SilcsBio GUI to set up, run, and analyze SSFEP calculations** as described in *Graphical User Interface Quickstart*.

12.2.1 SSFEP simulation setup

To perform the SSFEP precomputation simulations, protein coordinates in PDB file format and parent ligand coordinates in mol2 file format are required. The protein should have termini properly capped, missing loops built or the ends of the missing loops capped, standard atom and residue names, and sequential atom and residue numbering. Using these two files, run the following:

```
${SILCSBIODIR}/ssfep/1_setup_ssfep prot=<Protein PDB> lig=<Ligand Mol2/  
↪SDF>
```

Warning: The setup program internally use the GROMACS utility `pdb2gmh`, which may have problems processing the protein PDB file. The most common `pdb2gmh` issue involves mismatches between the expected residue name/atom names in the input PDB and those defined in the CHARMM force-field.

To fix this problem: Run the `pdb2gmh` command manually from within the `1_setup` directory for a detailed error message. Please contact support@silcsbio.com for additional assistance.

Following completion of the setup, run 10 MD jobs:

```
${SILCSBIODIR}/ssfep/2_run_md_ssfep prot=<Protein PDB> lig=<Ligand_  
↪Mol2/SDF>
```

This command will submit 10 jobs to the pre-defined queue: 5 for the ligand in water and 5 for the ligand complexed with protein.

Once the precomputation simulations are completed, the `2_run_md/1_lig/[1-5]` and `2_run_md/2_prot_lig/[1-5]` directories will contain `*.1-10.whole.trr` trajectory

files. If these files are not generated, then your simulations are either still running or have stopped due to a problem. Look into the log files within these directories for an explanation of the failure.

12.2.2 Chemical group transformations

Create a text file `modifications.txt` with instructions listing the desired modifications to the parent ligand.

Three types of modifications can be performed, as listed in the table.

Command	Modification
JOIN	Join a fragment (mol2) to the parent at a defined site
REPL	Replace an atom at a defined site on the parent with a fragment (mol2)
MUDE	Change an atom at a defined site on the parent to another atom-type

Table 12.1: Available modification types.

An example ligand modification input file is provided in `examples/ssfep/modification.txt` file. For help on how to define these operations, run the following command:

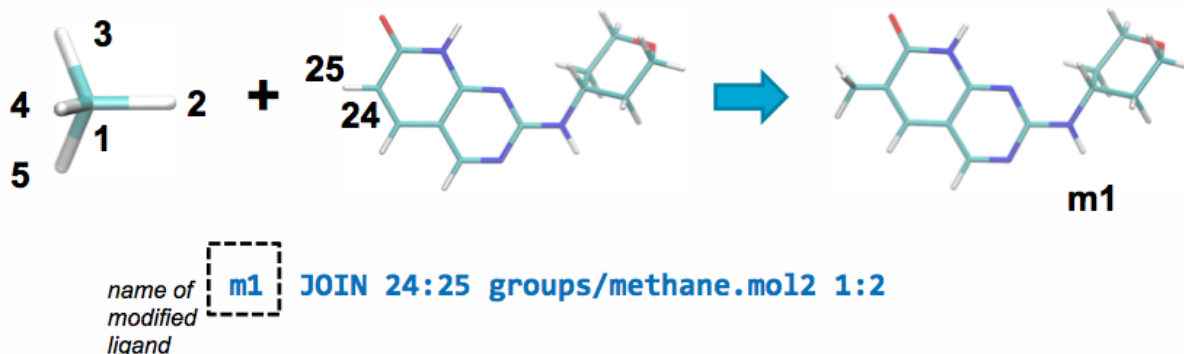
```
${SILCSBIODIR}/programs/chemmod -h
```

Access the groups directory from the following location to look at all the possible fragments that can be added/joined to the parent ligand:

```
${SILCSBIODIR}/data/groups
```

Modifications JOIN example

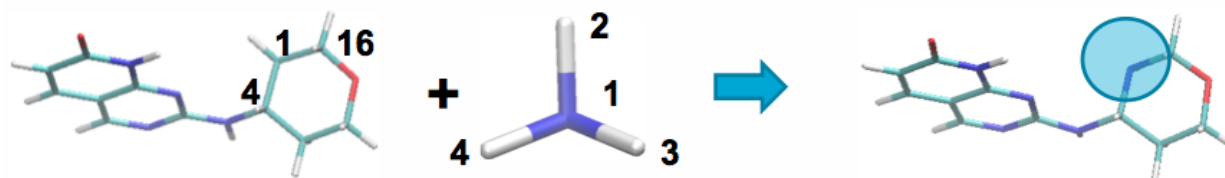
A **JOIN** operation can be performed between the parent and a `ch4.mol2` fragment by adding the following line to `modifications.txt`:



This line will join atom 24 in the parent ligand with atom 1 in `methane.mol2` and delete atoms 25 and 2 in the parent ligand and the joined fragment, respectively.

Modifications REPL example

A **REPL** operation can be performed between the parent and a `ch4.mol2` fragment by adding the following line to `modifications.txt`:



```
m1  REPL 1:4:16 groups/nh3.mol2 1:2:3
```

This line will replace atom 1 of the parent ligand with atom 1 of the fragment by aligning atoms 2 & 3 of the fragment with atoms 4 & 16 of the parent, respectively, and replacing the carbon in the ring with a nitrogen atom.

Modifications MUDE example

The same transformation in the previous section can also be achieved using a **MUDE** operation:

```
m2  MUDE MU 1:7 DE 21
```

This line will mutate atom 1 (atom index number) in the parent with nitrogen (atom index number 7) along with deleting the hydrogen (atom index number 21) attached to the parent carbon.

Ligand decoration

To evaluate multiple modifications to a single site on the parent ligand, use the following syntax:

```
m1  JOIN 24:25 ssfep_lig_decoration_master_modification.txt
```

Copy `ssfep_lig_decoration_master_modification.txt` from the `${SILCSBIODIR}/data/` folder. This master modification list contains 70 commonly-used modifications. Be careful to pay attention to chemistry; if some of these modifications are not suitable for a site, you can comment them out using `!` at the beginning of that line.

This single entry in your `modifications.txt` will generate 70 separate modifications to the parent ligand, each with a prefix `m1_`

12.3 Evaluating binding affinity changes

Once `modifications.txt` has been prepared and the MD simulations involving the parent ligand are completed, run the following script to set up a $\Delta\Delta G$ calculation.

```
${SILCSBIODIR}/ssfep/3a_setup_modifications prot=<Protein PDB> lig=
  ↳<Ligand Mol2/SDF File> mod=modifications.txt
```

This will submit 10 jobs to evaluate all snapshots from the completed MD simulations of the parent ligand in order to calculate the change in free energy for every modification specified in your `modifications.txt`. Structures of these modifications in mol2 format are output as `3_analysis_<modified ligand name entry in modifications.txt>/mod_files/*.mol2`.

After these jobs complete, you may obtain $\Delta\Delta G$ for your full list of modifications using:

```
${SILCSBIODIR}/ssfep/3b_calc_ddG_ssfep mod=modifications.txt
```

Example output follows:

m1	<chem>c1(cc2cc(ccc2[nH]c1=O)NS(=O)(=O)c1ccccc1)C</chem>	-2.7
name of the modified ligand	SMILES string of the modified ligand	$\Delta\Delta G$ for the modification

CGENFF: CHARMM GENERAL FORCE FIELD

13.1 Background

The CHARMM General Force Field (CGenFF) covers a wide range of chemical groups present in biomolecules and drug-like molecules, including a large number of heterocyclic scaffolds [1]. The parametrization philosophy behind the force field focuses on quality at the expense of transferability, with the implementation concentrating on an extensible force field.

CGenFF uses the CHARMM additive potential energy function to calculate the energy as a function of the Cartesian coordinates of the system, as shown below.

$$\begin{aligned}
 &\text{Intramolecular (internal, bonded terms)} \\
 &\sum_{\text{bonds}} K_b (b - b_0)^2 + \sum_{\text{angles}} K_\theta (\theta - \theta_0)^2 + \sum_{\text{dihedrals}} K_\phi (1 + \cos(n\phi - \delta)) \\
 &+ \sum_{\text{improper}} K_\psi (\psi - \psi_0)^2 + \sum_{\text{Urey-Bradley}} K_{\text{UB}} (r_{1,3} - r_{1,3;0})^2 \\
 &\text{Intermolecular (external, nonbonded terms)} \\
 &\sum_{\text{nonbonded}} \frac{q_i q_j}{4\pi D r_{ij}} + \epsilon_{ij} \left[\left(\frac{R_{\text{min},ij}}{r_{ij}} \right)^{12} - 2 \left(\frac{R_{\text{min},ij}}{r_{ij}} \right)^6 \right]
 \end{aligned}$$

The bonded or intramolecular part of the potential energy function consists of terms for the bonds, valence angles, torsion or dihedral angles, improper dihedral angles, and a Urey-Bradley term, where b_0 , θ_0 , ϕ_0 , and $r_{1,3;0}$, respectively, are the bond, angle, improper and Urey-Bradley equilibrium values, the K 's are the corresponding force constants, and n and δ are the dihedral multiplicity and phase. The nonbonded or intermolecular portion consists of an electrostatic term, with q_i and q_j being the respective partial atomic charges on atoms i and j , and a van der Waals (vdW) term, which is treated by the Lennard-Jones (LJ) 6-12 potential in which ϵ_{ij} is the well depth, $R_{\text{min},ij}$ is the radius, and r_{ij} is the distance between i and j .

It is apparent that a simulation on any system of practical interest requires large numbers of parameters. To make the assignment of these parameters practical, force fields require atom types to be assigned to all the atoms in the system, with the parameters associated with combinations of atom types. For instance, the parameter list will contain K_ϕ , n , and δ values for the dihedral parameters

associated with all combinations of four atom types that occur in the molecules supported by the force field. Thus, the first step of assigning parameters for a chemical system is assigning atom types to that system.

CGenFF comes with an algorithm that can automatically assign atom types to a given molecule. The atom typer is rule-based and programmable, making it easy to implement complex atom typing rules and to update the atom typing scheme as the force field grows. Assignment of bonded parameters is based on substituting atom types in the definition of the desired parameter. A penalty is associated with every substitution and the existing parameter with the lowest total penalty is chosen as an approximation for the desired parameter. Charges are assigned using an extended bond-charge increment scheme that is able to capture inductive and mesomeric effects across up to 3 bonds. More details can be found in [2].

13.2 Usage

The CGenFF program accepts Mol2 format files. For correct atom typing, it is important that all hydrogens are present, the system has correct protonation and tautomeric states, and that the bond orders are correct.

Once the Mol2 file is ready, the CGenFF program can be executed:

```
${SILCSBIODIR}/programs/cgenff <mol2file>
```

This produces a stream file in standard output. The output can be redirected to a `.str` file. The stream file consists of a topology file and the extra parameter file combined in a single output. It is up to the user to whether to use the stream file directly or to split the file into two separate files.

As an example, the adrenalin molecule is parametrized in CGenFF. The example input and output files can be found in `${SILCSBIO}/examples/cgenff/adrenalin.mol2` and `${SILCSBIO}/examples/cgenff/adrenalin.str`, respectively.

The output stream file has two major sections.

```
* Toppar stream file generated by
* CHARMM General Force Field (CGenFF) program version 1.0.0
* For use with CGenFF version 3.0.1
*
read rtf card append
... topology section ...

END

read param card flex append
... parameter section ...
```

(continues on next page)

(continued from previous page)

```
END
RETURN
```

In CHARMM, asterisk and exclamation marks represents comments. If you wish to separate topology and parameter sections, you can take out the corresponding section after `read rtf` or `read param` to the `END` statement. Let's take a look at topology section:

```
RESI ADNR          0.000 ! param penalty= 113.000 ; charge penalty=
→16.607
GROUP              ! CHARGE    CH_PENALTY
ATOM O1            OG311  -0.649 !    11.757
ATOM O2            OG311  -0.530 !    0.000
ATOM O3            OG311  -0.530 !    0.000
ATOM N             NG311  -0.524 !   16.607
ATOM C1            CG311   0.127 !   16.171
```

Above is the beginning of the topology section. This section defines the atom names and types, as well as the atomic charges. Each entry is followed by a charge penalty score. The initial atomic charges are assigned based on chemical analogy to existing parametrizations in the force field. The penalty score becomes high when a good analogy is not found.

Below is an excerpt from the parameter section. When an exact parameter is not found in an existing parameter database, an entry is added based on an analogous parameter. Similar to atomic charges, each entry in parameter is also followed by a penalty score.

```
ANGLES
CG2R61 CG311  OG311    75.70    110.10 ! ADNR , from CG2R61 CG321
→OG311, penalty= 4
CG311  CG321  NG311    43.70    112.20 ! ADNR , from CG331 CG321 NG311,
→ penalty= 1.5
CG321  NG311  CG331    40.50    112.20    5.00    2.42170 ! ADNR , from
→CG3AM1 NG311 CG3AM1, penalty= 35
```

High penalty scores indicate that a parameter may be targeted for further optimization. Typically, penalty scores greater than 50 warrant further optimization.

13.3 GROMACS-readable parameters

To get parameters in GROMACS format, execute the following command.

```
${SILCSBIODIR}/cgenff/cgenff_to_gmx mol=<mol2file>
```

This command produces three files `<mol2file>_gmx.top`, `<mol2file>_gmx.pdb` &

<mol2file>_charmm.str. <mol2file>_gmx.top/pdb contain the GROMACS readable parameters and the coordinate information. <mol2file>_charmm.str contain CHARMM readable parameters.

13.4 CGenFF Parameter Optimizer

The CGenFF program performs atom typing and assignment of parameters and charges by analogy in a fully-automated fashion. Atom typing is done by a deterministic programmable decision tree. Assignment of bonded parameters is based on substituting atom types in the definition of the desired parameter. A penalty is associated with every substitution and the existing parameter in the current version of the force field with lowest total penalty is chosen as an approximation for the desired parameter. The penalty score is returned to the user as an indication of the approximation needed to obtain the desired parameter. For example, dihedral parameters with higher penalties (typically >200) may be candidates for further optimization. When using the Optimizer, the atom typing and parameter assignment is performed internally such that the user only needs to supply the Mol2 file of the molecule of interest.

CGenFF Parameter Optimizer allows for automatic optimization of rotatable dihedrals. Once the user specifies the dihedral to be optimized, the Optimizer coordinates the generation of quantum mechanical (QM) target data followed by the fitting of force field parameters to these target data. The Optimizer will first spawn [Psi4](#) QM jobs. It will then collate the resulting QM dihedral scan data. Finally, it will fit force field parameters to these data using the least-square fitting procedure [LSFitPar](#) and output the new, optimized force field parameters. If multiple dihedrals are to be optimized, then each dihedral is fitted separately targeting independent QM scans on each dihedral. During the fitting, the initial multiplicities are those assigned by the CGenFF program. Once the initial fit is complete and the RMS error determined, the program automatically refits the data using a multiplicity of 1, then multiplicities of 1 and 2, 1, 2 and 3 and 1, 2, 3 and 6. If improvements in the RMSE are obtained beyond a cutoff (default is 10% of the RMSE) with the additional multiplicities, then those parameters are selected.

The Optimizer accepts molecules in Mol2 file format, with the following requirements: hydrogens must be explicitly defined, ionizable groups must have correct protonation states, and bond-order between atoms must be correctly defined.

To run the program, set the following environment variables,

```
export SILCSBIODIR=<silcsbio>
export PSI4DIR=<psi4>
export GMXDIR=<gromacs/bin>
```

The Psi4 package can be obtained from the [Psi4 download page](#). For the easiest installation, use the Psi4 Binary Installer, run the command:

```
./Psi4conda-latest-py36-Linux-x86_64.sh
```

and following the step-by-step guide.

After Psi4 has been installed, set the `PSI4DIR` variable correctly and proceed with using the optimizer. To make sure `PSI4DIR` is correctly set, check if `$PSI4DIR/bin/psi4` is accessible.

Note that downloading the Psi4 source code and compiling the program locally with the appropriate switches can lead to a significant gain in performance over the downloaded binary version.

13.4.1 Usage

```
${SILCSBIODIR}/cgenff-optimizer/optimize_cgenff_par mol=<mol2file>
```

The second line of the Mol2 file contains a string naming the molecule. Certain molecular modeling programs will put the string “*” on this line instead of a descriptive name for the molecule. In such cases, please replace the string with an alphanumeric string, else the Optimizer will fail.

Along with the required argument of `mol=<mol2file>`, the following additional parameters can also be set:

1. Penalty score :

```
penalty=<score; default 50>
```

By default, the program identifies rotatable dihedrals with penalties greater than 50 for optimization. This can be changed by adjusting the `penalty` argument, to increase/decrease the list of dihedrals that will be optimized.

2. Dihedral step size :

```
dihedral_step_size=<step size; default 15>
```

Each dihedral identified for optimization is rotated through 360 degrees with a step-size of 15 degrees to generate QM target data. This step-size can be changed by adjusting the `dihedral_step_size` argument to increase or decrease the resolution of the dihedral scan.

3. Number of cores used for QM optimization:

```
nproc=<number of core; default all available cores on_
↪workstation/8 cores on HPC>
```

At each scan point for each dihedral, a constrained QM optimization is performed. Psi4 can make use of multiple CPU cores to run this optimization more quickly. When the Optimizer is installed on a high-performance computing cluster (HPC), separate jobs are submitted for each of the identified dihedrals simultaneously, and each job requests `nproc` cores. When the Optimizer is installed on a workstation, then each scan point for each dihedral is run one after the other using all available cores on the workstation. This default behavior can be changed using the `nproc` argument.

4. Molecular-orbital theory:

```
mo_theory=<MP2/HF; default MP2>
```

Constrained QM optimization for each dihedral at each scan point is performed using Møller–Plesset perturbation theory (MP2, specifically the Psi4 DF-MP2/6-31G(d) model chemistry). If the user wants to instead use Hartree-Fock (HF), then it can be set using `mo_theory`.

5. Energy difference cutoff to eliminate rotamers from QM optimization and subsequent fitting:

```
dg_cutoff=<energy_difference; default 100.>
```

The optimizer initially generates all the rotamers for each dihedral being optimized as required for the potential energy surface (PES) and performs a CGenFF energy optimization on each restrained rotamer. If the relative energy of each rotamer is greater than the `dg_cutoff` value (kcal/mol), that rotamer is omitted from the QM optimization and subsequent parameter fitting. This is performed to eliminate rotamers with steric clashes that can be problematic for the QM optimization as well as lead to the parameter optimizer attempting to fit to high energy regions of the PES that are typically not sampled in MD simulations.

6. Specification of compute node for job submission:

```
hpc=<true/false; default true>
```

Installation of the CGenFF optimizer specifies the computer on which the jobs will be run, which is typically a local HPC cluster such that the default is `hpc=true`. However, if the user wants to run the job on their local workstation then `hpc=false` may be specified. Alternatively, if the default is false, `hpc=true` can be specified to run the job on the HPC cluster. This requires that the appropriate cluster be specified in the default installation.

7. Running jobs sequentially or in parallel when `hpc=true`.

```
jobtype=<seq/par; default seq>
```

When the QM jobs are submitted to an HPC queue they will run sequentially using the specified number of processors (`nproc`). However, if adequate compute resources are available it is possible to have all the QM jobs run simultaneously in parallel by specifying `jobtype=par` with each job using the specified number of processors (`nproc`). Care should be taken when using this option as the number of individual QM jobs can be quite large ($n \times 24$ jobs where n is the number of dihedral parameters being optimized). However, use of the option can lead to the QM jobs finishing rapidly.

8. Setting the RMS energy difference to not redo the least-squares fitting using different multiplicities.

```
rmse_cutoff=<RMS energy cutoff to determine if additional_
↳multiplicities should be tested; default=0.0>
```

The initial least-squares fit applies the multiplicities in the original CGenFF parameters assigned to the dihedral being optimized. From the fit the RMS difference between the target QM and optimized MM parameters is calculated. If that value is greater than `rmse_cutoff` then least-squares fitting is performed with additional multiplicities. `rmse_cutoff` is set to zero by default to force the additional multiplicities to be included in the least-squares fitting. Note that the fitting may be repeated using the calculated QM data as described in the “Practical Considerations” section below.

13.4.2 Example Usecase

The following demonstrates usage of the CGenFF Parameter Optimizer. The input file `ethene_co_isoxazole.mol2` is available in `${SILCSBIODIR}/examples/cgenff/`. When running the Optimizer it is suggested that a subdirectory be created for each molecule and the job run from within that subdirectory.

```
${SILCSBIODIR}/cgenff-optimizer/optimize_cgenff_par mol=ethene_co_
↳isoxazole.mol2
```

The resulting output is a CHARMM-compatible CGenFF stream file named `ethene_co_isoxazole_optimized.str`. Since this molecule does not contain any dihedral parameter with penalty greater than 50, running the above command results in:

```
Nothing to optimize based on the penalty score threshold of 50
```

The user can look through the output stream file to identify dihedral parameters that may benefit from optimization:

```
DIHEDRALS
CG2DC3 CG2DC1 CG2O5 CG2R51      1.4000  2   180.00 ! NONAME.* , from_
↳CG2DC3 CG2DC1 CG2O5 OG2D3, penalty= 26.5
HGA4   CG2DC1 CG2O5 CG2R51      0.0000  2   180.00 ! NONAME.* , from_
↳HGA4 CG2DC1 CG2O5 OG2D3, penalty= 26.5
CG2DC1 CG2O5 CG2R51 CG2R51      1.5850  2   180.00 ! NONAME.* , from_
↳CG2O3 CG2O5 CG2R61 CG2R61, penalty= 46.5
CG2DC1 CG2O5 CG2R51 OG2R50      1.5850  2   180.00 ! NONAME.* , from_
↳CG2O3 CG2O5 CG2R61 CG2R61, penalty= 49
OG2D3  CG2O5 CG2R51 CG2R51      1.5850  2   180.00 ! NONAME.* , from_
↳OG2D3 CG2O5 CG2R61 CG2R61, penalty= 33.5
OG2D3  CG2O5 CG2R51 OG2R50      1.5850  2   180.00 ! NONAME.* , from_
↳OG2D3 CG2O5 CG2R61 CG2R61, penalty= 13
CG2O5  CG2R51 CG2R51 CG2R52      0.0000  2   180.00 ! NONAME.* , from_
↳CG2O1 CG2R51 CG2R51 CG2R52, penalty= 3
```

(continues on next page)

(continued from previous page)

```
CG2O5  CG2R51 CG2R51 HGR51      1.0000  2   180.00 ! NONAME.* , from_
→CG2R51 CG2R51 CG2R51 HGR51, penalty= 16.5
CG2O5  CG2R51 OG2R50 NG2R50     8.5000  2   180.00 ! NONAME.* , from_
→CG2R51 CG2R51 OG2R50 NG2R50, penalty= 16.5
```

If the user now wants to fit CG2DC1 CG2O5 CG2R51 OG2R50, which has a penalty score of 49, the program can be re-run with a lowered penalty threshold of 48:

```
${SILCSBIODIR}/cgenff-optimizer/optimize_cgenff_par mol=ethene_co_
→isoxazole.mol2 penalty=48
```

This will result in:

```
#####
for dihedral = CG2DC1 CG2O5 CG2R51 OG2R50, parameter penalty = 176.
→000000;
#####
```

Will be optimizing the parameters of the above listed dihedral(s)

Note that all parameters associated with dihedrals about a rotatable bond with a penalty score exceeding the penalty threshold will be optimized. Dihedrals that are considered rigid based on being located in rings, being double bonds and so on, are excluded from fitting. This selection process can lead to a situation in which two or more sets of dihedral parameters associated with the same rotatable bond are being fit independently. While each of those fits may appear to be successful, when those parameters are combined and applied to the molecules of interest, the conformational energy for rotation about that bond will likely be inaccurate. Such a situation should be avoided by setting the penalty tolerance to be higher than the penalty values for those parameters and one of the dihedrals selected for optimization as described in the following section.

If the user wants to fit the parameters for one or more specific dihedrals if none are selected based on the penalty score, or in addition to the automatically selected dihedrals, the user can manually supply the four atoms defined each specific dihedral on prompt:

```
${SILCSBIODIR}/cgenff=optimizer/optimize_cgenff_par mol=ethene_co_
→isoxazole.mol2 penalty=200
```

will result in :

```
CHARMM General Force Field (CGenFF) program version 2.1.0
released the 27th of October 2016
Copyright (C) 2017 SilcBio LLC
and University of Maryland, School of Pharmacy. All Rights Reserved.

Now processing molecule sulf ...
```

(continues on next page)

(continued from previous page)

```
#####
#####

Nothing to optimize based on the penalty score threshold of 50

Do you want to perform optimization with anymore dihedrals? [Y/N; _
↪default N]
```

To add to the list, type Y, and then supply dihedral(s) with the format AtomType1 AtomType2 AtomType3 AtomType4. The program then summarizes the updated-list:

```
List updated; will be attempting optimization with the following _
↪dihedrals

#####
CG2DC1 CG2O5 CG2R51 OG2R50
#####
```

The last column of the PDB file <mol>_cgenff_atomtypes.pdb created by the Optimizer identifies the atom type for each atom in the system which may be inspected to select specific parameters for optimization. For the current example, from the information in ethene_co_isoxazole_cgenff_atomtypes.pdb, atom 5 has the atom type CG2R51:

ATOM	1	C	NONA	1	0.759	0.680	1.188	1.00	0.00	↪
↪	CG2R52									
ATOM	2	H	NONA	1	1.645	1.222	1.497	1.00	0.00	↪
↪	HGR52									
ATOM	3	C	NONA	1	-0.353	1.220	0.530	1.00	0.00	↪
↪	CG2R51									
ATOM	4	H	NONA	1	-0.508	2.244	0.227	1.00	0.00	↪
↪	HGR51									
ATOM	5	C	NONA	1	-1.205	0.177	0.349	1.00	0.00	↪
↪	CG2R51									
ATOM	6	C	NONA	1	-2.542	0.163	-0.296	1.00	0.00	↪
↪	CG2O5									
ATOM	7	C	NONA	1	-3.305	-1.129	-0.378	1.00	0.00	↪
↪	CG2DC1									
ATOM	8	H	NONA	1	-2.855	-2.041	0.043	1.00	0.00	↪
↪	HGA4									
ATOM	9	C2	NONA	1	-4.530	-1.255	-0.944	1.00	0.00	↪
↪	CG2DC3									
ATOM	10	H21	NONA	1	-5.050	-2.226	-0.980	1.00	0.00	↪
↪	HGA5									
ATOM	11	H22	NONA	1	-5.045	-0.383	-1.387	1.00	0.00	↪
↪	HGA5									

(continues on next page)

(continued from previous page)

ATOM	12	O1	NONA	1	-3.016	1.204	-0.756	1.00	0.00	└
→	OG2D3									
ATOM	13	O	NONA	1	-0.636	-0.949	0.875	1.00	0.00	└
→	OG2R50									
ATOM	14	N	NONA	1	0.613	-0.617	1.406	1.00	0.00	└
→	NG2R50									

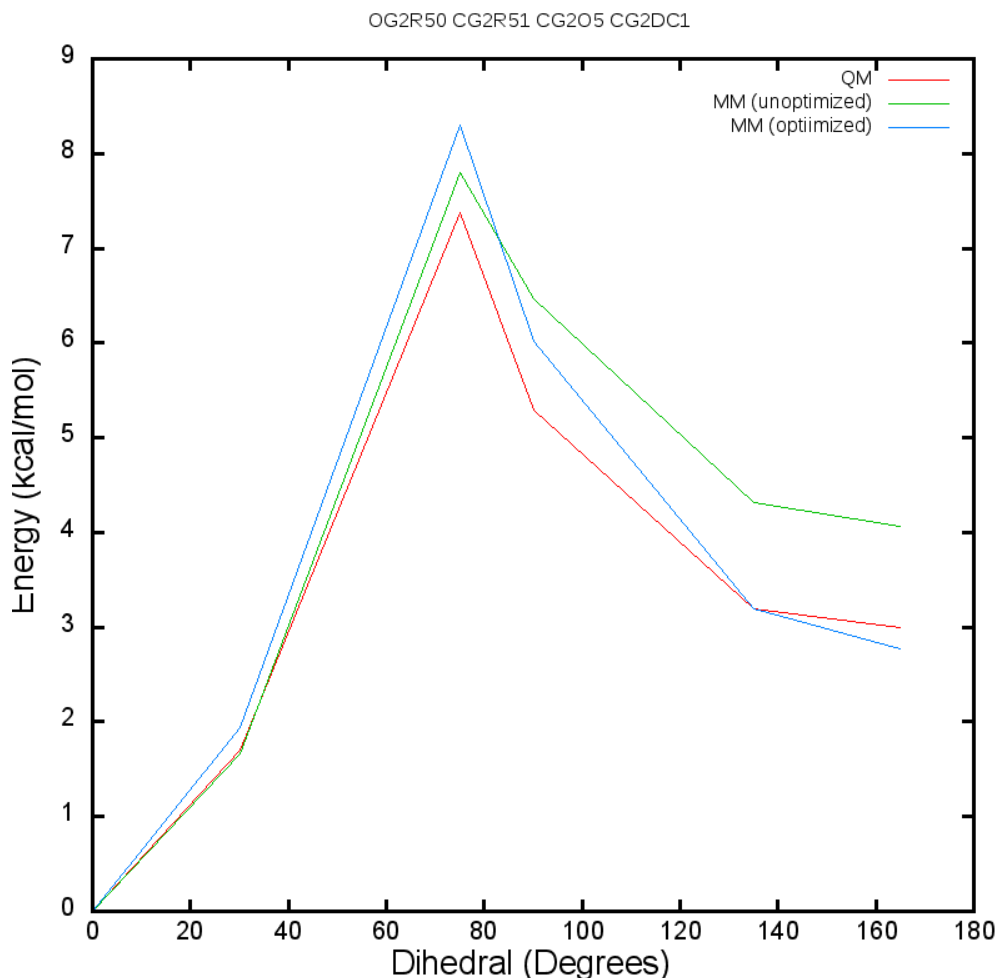
Following the identification of the dihedrals to the fitted, the Optimizer performs a complete molecular mechanics (MM) minimization of the molecule using the default CGenFF force field parameters. Next, each identified dihedral is independently rotated through 360 degrees with the specified step-size. Separate Psi4 jobs for constrained QM optimization around the specified dihedral are then performed either serially, one-by-one when running on a workstation, or in parallel when running on a HPC cluster.

The program waits on the QM jobs to finish. After the completion of the QM jobs, the MM energy is computed for each scan point. Both the QM and MM energies are then used by the lsfitpar program to fit the dihedral parameter. Updated parameters are output as `<mol>_optimized.str`.

Graphs of the dihedral scan energies are output in the files `qm_mm_*.png`. If the computing system where you are running the Optimizer does not contain gnuplot or gnuplot linked against the png library, you may copy the `4plot` directory to a computing system that does and run the gnuplot files there.

Note that the most robust test of the optimized parameters involves testing them directly in a molecular modeling package with the new parameters added to the CGenFF parameter file (see below).

The following is a graph produced by the Optimizer for the `ethene_co_isoxazole` example and demonstrates the better agreement with the QM data following dihedral parameter optimization:



The default CGenFF parameter for dihedral CG2DC1 CG2O5 CG2R51 OG2R50 was

CG2DC1	CG2O5	CG2R51	OG2R50	1.5850	2	180.00	! RMSE = 0.468999
--------	-------	--------	--------	--------	---	--------	-------------------

and the replacement parameter produced by the Optimizer is

CG2DC1	CG2O5	CG2R51	OG2R50	0.5728	2	0.00	! RMSE = 0.111323
--------	-------	--------	--------	--------	---	------	-------------------

13.4.3 Performing dihedrals optimization in the background

The above example runs the optimization on the command line requiring the terminal in which the job is submitted to remain open. To run the optimization in the background, the 'nohup' command may be used as in the following example. Note that a small script would be required to identify specific dihedrals for optimization.

```
nohup $SILCSBIODIR/cgenff-optimizer/optimize_cgenff_par.sh mol=ethene_
→co_isoxazole.mol2 > cgenff_opt_run1.txt &
```

13.4.4 Including new dihedral parameters with CGenFF

Currently, the optimized parameters generated by the CGenFF optimizer are NOT automatically added to the CGenFF parameter database. Accordingly, the user needs to manually add the parameters to the file using a standard editor (e.g., vi or emacs).

```
$SILCSBIODIR/data/par_all36_cgenff.prm
```

In the editor go to the DIHEDRALS section of the parameter file and then manually add the new parameters into the file. The new parameters are in filename_optimized.str found in the directory in which the optimization was run. “filename” corresponds to the molecule filename.mol2 used to initiate the optimization. The optimized dihedral are at the end of the DIHEDRALS section of that file and are indicated by the comment containing the RMSE values obtained from the fitting of each dihedral parameter as follows.

CG2R51	CG2R51	NG311	CG331	3.8499	2	180.00	! RMSE = 0.
→58618							
CG2R51	CG2R51	NG311	CG331	1.5145	3	180.00	! RMSE = 0.
→58618							
CG3C52	CG2R51	NG311	CG331	2.1370	2	180.00	! RMSE = 0.
→245345							
CG3C52	CG2R51	NG311	CG331	1.4058	3	180.00	! RMSE = 0.
→245345							

Those parameters may be cut and paste directly into par_all36_cgenff.prm. Note that the file is generally protected and system administrator assistance is required to update this file. In the case of replacing dihedral parameters that are already in par_all36_cgenff.prm, the original parameters should be commented with a ! and the new parameters added.

13.4.5 Additional output information

Upon completion of the optimization the data generated is organized and placed in the subdirectory “raw_data” in which there is an additional subdirectory “psi4_calc” that contains files from the QM calculations.

- optimize_cgenff_par.log

Summary of the dihedrals being optimized and the optimization process including the RMSE data and final parameters.

- dih_params_to_optimize*.txt

Dihedral parameters initially selected and updated list targeted for optimization.

- `params*.{inp,out}`

Inputs and outputs of MM minimizations to calculate the potential energy surfaces. Includes information on highly unfavorable conformations that were not included in the final parameter fitting.

- `params_aft_qm.out`

Outputs of MM minimizations using the optimized parameters to calculate the potential energy surface.

- `lsqfit_op_0*.{inp,out}`

Inputs and outputs for the parameter least-squares fitting using the original multiplicity along with fits using alternate multiplicities (`grep "Final RMSE" *.out` to see RMSE for all runs).

- `mm*.prm`

Final parameters from the fits with the different multiplicities.

- `*.dat`

Files containing dihedral angles and energies for fitting. Note that the `mm_init_ener_0.dat` is the MM PES with the targeted dihedral parameters set to zero.

13.4.6 Practical considerations

The use of the CGenFF penalty scores to select dihedrals for optimization in conjunction with the selection of only rotatable bonds is designed for an automated approach to dihedral parameter optimization. However, this may often lead to multiple dihedrals being selected for optimization, which is computationally demanding, or no dihedrals being selected based on low or zero penalty scores, where zero indicates that the specific parameter is already in the CGenFF force field and, by default, such dihedrals will not be listed as available for optimization. However, in cases where the user is concerned about the accuracy of a particular rotatable bond even when it is already in the CGenFF parameter set (e.g., a rotatable bond involved in the link between two ring systems that are part of a lead compound), then the user may specifically identify the atom types defining that dihedral parameter(s) and input them individually. While the penalty scores represent a useful metric by which to judge the quality of a dihedral parameter it should be noted that the CGenFF penalty scores are based on analogy to known parameters. Accordingly, a parameter with a high penalty may yield acceptable conformational energies, while a parameter already in CGenFF (i.e., penalty = 0) may not yield an acceptable energy surface due to, for example, the terminal rings about the associated bond creating a significantly different context than that in which the dihedral parameter was initially optimized.

An important consideration is molecular size. As the fitting procedure requires QM calculations the size of the molecule under study significantly impacts the speed of the overall fitting procedure.

To limit the computational demand while achieving the desired accuracy in the parameters it is suggested that compounds be subdivided into model compounds extracted from that full compound that contain two terminal ring systems along with the linker between those rings. The number of substituents on each ring should be limited to those deemed essential to represent the chemical character of the rings while minimizing the number of substituents to limit computational costs. For example a compound with three ring systems with two linkers would be subdivided into two model compounds with two rings each, with one of the rings common to both model compounds. Once the two individual CGenFF-optimizer runs are finished, the new parameters may be combined and added to your the CGenFF parameter files.

During parameter fitting, the program initially uses the dihedral parameter multiplicities assigned by the CGenFF program. Once the RMSE is returned, the program then does additional fitting with a multiplicity of 1, then multiplicities of 1 and 2, 1, 2 and 3 and 1, 2, 3 and 6. The current implementation outputs the results from all the multiplicities tested. As the lowest RMSE will generally be with multiplicities 1,2,3,6 the user may select a set of parameters with different multiplicities. Those parameters are in the files `raw_data/mm*.prm`. Note that the multiplicities of 4 and 5 are omitted as these are rarely used for rotatable bonds even though, in some cases, the CGenFF program will assign dihedral parameters with these multiplicities and the program will initially include a dihedral with a multiplicity of 4 or 5 in the initial fitting.

If the level of agreement using the final parameters is not satisfactory it suggests hysteresis in the energy surface. To overcome this the user may consider focusing the fitting on an ‘appropriate’ part of the energy surface by trimming down some data-points on the QM & MM energies to get a better fit to the low energy region of the energy surface. To achieve this consider running `${SILCSBIODIR}/cgenff-optimizer/refit_par_with_subsurface dih_qm_<dihno>.dat dih_mm_<dihno>.dat` (Under development).

Upon completion of the fitting a subdirectory ‘raw_data’ is created that contains an extensive number of data file with the various QM energies, MM energies and additional information. In addition, the subdirectory ‘raw_data/psi4_calc’ contains the inputs and outputs from the psi4 QM optimizations. These files may be used for additional fitting or analysis.

In cases where the least-squares fitting is not completed successfully, possibly due to all the QM jobs not finishing, fitting alone may be performed if all the QM data is directly available in subdirectory `psi4_calc` (not `raw_data/psi4_calc`). For example, if a subset of the QM jobs did not finished the job input scripts in `psi4_calc` (e.g., `psi4_inp_xxxx_1_23.inp`) maybe submitted directly to complete those jobs followed by resubmission of the `cgenff-optimizer` command from the parent directory.

CHAPTER
FOURTEEN

REFERENCES

BIBLIOGRAPHY

- [1] K Vanommeslaeghe, E Hatcher, C Acharya, S Kundu, S Zhong, J Shim, E Darian, O Guvench, P Lopes, I Vorobyov, and Alexander D MacKerell Jr. CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *J. Comput. Chem.*, 31(4):671–690, March 2010.
- [2] Kenno Vanommeslaeghe and Alexander D MacKerell Jr. Automation of the CHARMM General Force Field (CGenFF) I: bond perception and atom typing. *J. Chem. Inf. Model.*, 52(12):3144–3154, December 2012.
- [3] You Xu, Kenno Vanommeslaeghe, Alexey Aleksandrov, Alexander D MacKerell Jr, and Lennart Nilsson. Additive CHARMM Force Field for Naturally Occurring Modified Ribonucleotides. *J. Comput. Chem.*, 37(10):896–912, April 2016.
- [4] Alexander D MacKerell Jr, Sunhwan Jo, Sirish Kaushik Lakkaraju, Christoffer Lind, and Wenbo Yu. Identification and Characterization of Fragment Binding Sites for Allosteric Ligand Design Using the Site Identification by Ligand Competitive Saturation Hotspots Approach (SILCS-Hotspots). *Biochim. Biophys. Acta. Gen. Subj.*, 1864(4):129519–129519, April 2020.
- [5] Olgun Guvench and Alexander D MacKerell Jr. Computational Fragment-Based Binding Site Identification by Ligand Competitive Saturation. *PLoS Comput. Biol.*, 5(7):e1000435–12, July 2009.
- [6] Sirish Kaushik Lakkaraju, E Prabhu Raman, Wenbo Yu, and Alexander D MacKerell Jr. Sampling of Organic Solutes in Aqueous and Heterogeneous Environments Using Oscillating Excess Chemical Potentials in Grand Canonical-like Monte Carlo-Molecular Dynamics Simulations. *J. Chem. Theory Comput.*, 10(6):2281–2290, June 2014.
- [7] Richard D Taylor, Malcolm MacCoss, and Alastair D G Lawson. Rings in drugs. *J. Med. Chem.*, 57(14):5845–5859, July 2014.
- [8] Marc O'Reilly, Anne Cleasby, Thomas G Davies, Richard J Hall, R Frederick Ludlow, Christopher W Murray, Dominic Tisi, and Harren Jhoti. Crystallographic screening using ultra-low-molecular-weight ligands to guide drug design. *Drug Discov. Today*, 24(5):1081–1086, May 2019.

- [9] Yang Zhang and Jeffrey Skolnick. SPICKER: a clustering approach to identify near-native protein folds. *J. Comput. Chem.*, 25(6):865–871, April 2004.
- [10] E Prabhu Raman, Wenbo Yu, Sirish K Lakkaraju, and Alexander D MacKerell Jr. Inclusion of multiple fragment types in the Site Identification by Ligand Competitive Saturation (SILCS) approach. *J. Chem. Inf. Model.*, 53(12):3384–3398, December 2013.
- [11] Vincent D Ustach, Sirish Kaushik Lakkaraju, Sunhwan Jo, Wenbo Yu, Wenjuan Jiang, and Alexander D MacKerell Jr. Optimization and evaluation of Site-Identification by Ligand Competitive Saturation (SILCS) as a tool for target-based ligand optimization. *J. Chem. Inf. Model.*, 59(6):3018–3035, April 2019.
- [12] Wenbo Yu, Sirish Kaushik Lakkaraju, E Prabhu Raman, Lei Fang, and Alexander D MacKerell Jr. Pharmacophore modeling using Site Identification by Ligand Competitive Saturation (SILCS) with multiple probe molecules. *J. Chem. Inf. Model.*, 55(2):407–420, February 2015.
- [13] E Prabhu Raman, Kenno Vanommeslaeghe, and Alexander D MacKerell Jr. Site-specific fragment identification guided by Single-Step Free Energy Perturbation calculations. *J. Chem. Theory Comput.*, 8(10):3513–3525, October 2012.
- [14] E Prabhu Raman, Sirish Kaushik Lakkaraju, Rajiah Aldrin Denny, and Alexander D MacKerell Jr. Estimation of relative free energies of binding using pre-computed ensembles based on the Single-Step Free Energy Perturbation and the Site Identification by Ligand Competitive Saturation approaches. *J. Comput. Chem.*, October 2016.
- [15] E Prabhu Raman, Wenbo Yu, Olgun Guvench, and Alexander D MacKerell Jr. Reproducing crystal binding modes of ligand functional groups using Site Identification by Ligand Competitive Saturation (SILCS) simulations. *J. Chem. Inf. Model.*, 51(4):877–896, April 2011.
- [16] Sirish Kaushik Lakkaraju, Wenbo Yu, E Prabhu Raman, Alena V Hershfeld, Lei Fang, Deepak A Deshpande, and Alexander D MacKerell Jr. Mapping functional group free energy patterns at protein occluded sites: nuclear receptors and G-protein coupled receptors. *J. Chem. Inf. Model.*, 55(3):700–708, March 2015.
- [17] Geoffrey A Heinzl, Weiliang Huang, Wenbo Yu, Bennett J. Giardina, Yue Zhou, Alexander D MacKerell Jr, Angela Wilks, and Fengtian Xue. Iminoguanidines as Allosteric Inhibitors of the Iron-Regulated Heme Oxygenase (HemO) of *Pseudomonas aeruginosa*. *J. Med. Chem.*, 59(14):6929–6942, June 2016.
- [18] Maryanna E Lanning, Wenbo Yu, Jeremy L Yap, Jay Chauhan, Lijia Chen, Ellis Whiting, Lakshmi S Pidugu, Tyler Atkinson, Hala Bailey, Willy Li, Braden M Roth, Lauren Hynicka, Kirsty Chesko, Eric A Toth, Paul Shapiro, Alexander D MacKerell Jr, Paul T Wilder, and Steven Fletcher. Structure-based design of N-substituted 1-hydroxy-4-sulfamoyl-2-naphthoates as selective inhibitors of the Mcl-1 oncoprotein. *Eur. J. Med. Chem.*, 113:273–292, May 2016.