



# Molecular Dynamics and Trajectory Analysis Procedure using the Schrödinger Simulation Suite

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# I. USING THE SCHRÖDINGER WORKSTATION

## A. LOGGING IN AND STARTING THE LICENSE

1. Log into the computer (username & password located in the computer lab)
2. Check to see if the ‘Configure Schrodinger Software’ window is already open, running, or minimized. That is the license server.
3. If it is not, follow these steps:
  - a. Right click on the desktop and click “Open Terminal”.
  - b. Type “`export SCHRODINGER=/opt/schrodinger/2019-2`” and press enter.
  - c. Type “`$SCHRODINGER/utilities/configure`” and press enter.
  - d. This will pull up a new window which will read ‘Licensing Your Software’.
  - e. Click on “Start Process” and the license will start running.
4. Once the license is started, open BioLuminate:
  - a. Right click on the desktop and open a new terminal.
  - b. Type “`./run_bioluminate.sh`” and enter.
  - c. Maestro BioLuminate will then open.

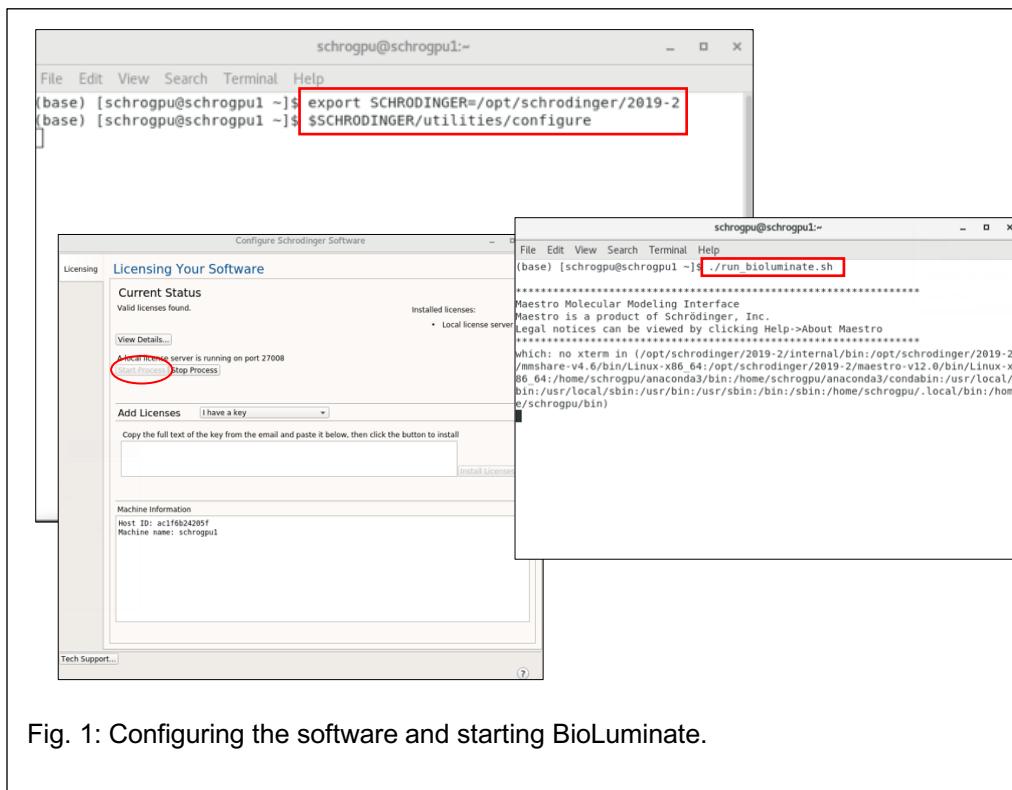
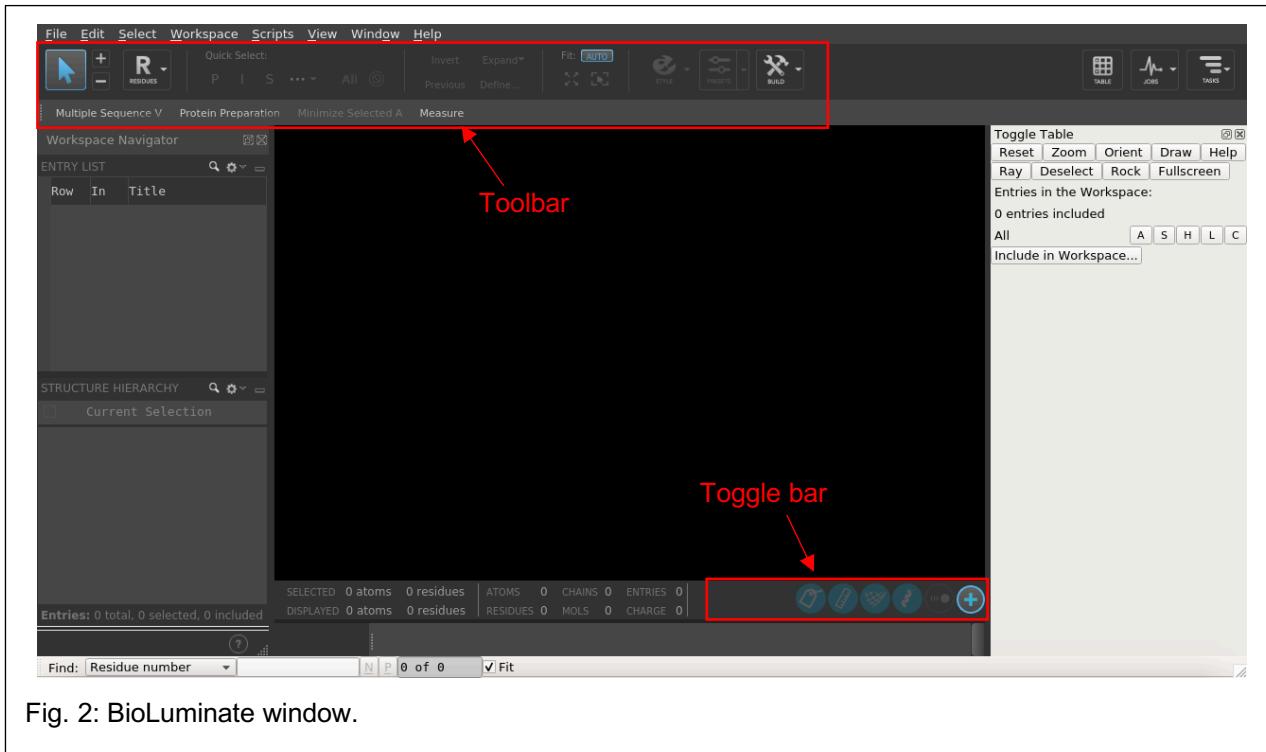


Fig. 1: Configuring the software and starting BioLuminate.

## B. NAVIGATION THROUGH BioLUMINATE

### ▪ OPENING A PROJECT AND SETTING YOUR WORKING DIRECTORY

1. To open or start a new project in BioLuminate, click on the **File** button in the upper left.
2. Then click on **New Project** or **Open Project** depending on whether you are starting a new project or opening one that you have already created.
3. Check your Working Directory by going to **File > Change Working Directory**
  - a. The Home Directory is the default working directory; however, it will be useful to create a folder with your name/ project name in Home to use as your working directory so that your files will be stored in that folder
4. To set your working directory, simply click on your folder in which you wish to save your files for your project and click choose
  - a. Make sure to **check your working directory every time** you use BioLuminate to ensure you are working in the correct location
  - b. If you forget to check your working directory and some of your files are saved in the Home Directory and you try to directly move your files into your own folder, you may encounter some problems. I will discuss this more in the **Troubleshooting** section.



- **MOUSE/KEYBOARD COMMANDS AND OTHER TOOLS**

*The following instructions assume you have entries in your project.*

1. In the **tool bar** found at the top of BioLuminate, there are options for **selection tools** and **setting the style** of your workspace.
  - a. You can hover above the tools to learn how to use them.
2. The **toggle bar** at the bottom of BioLuminate includes some other **workspace style settings** such as background colors and **displaying interactions** between molecules.
3. **To view an entry in your workspace**, click on the circle next to the entry's name.
  - a. If you want to view multiple entries at one time, for example to compare to peptide structures, hold down control and click on the circles next to the entries you want to view.
4. **To organize your workspace**, you may group entries of similar type by selecting the entries (highlighting them in the entry list), right click > **Group...**
  - a. Once you have a group, you may drag entries around in the list to organize.
5. **To zoom in/out** on an entry, hold the right side of the mouse down and drag the mouse towards and away from the computer.
6. **To move the entry around**, hold the scroll button on the mouse down and drag the mouse.
7. **To rotate your entry**, hold the left side of the mouse down and drag.
8. You may also use the **Toggle Table** found on the right side of the screen to move and rotate your entry as well as to reset the orientation of your entry.
9. **Ctrl + Space + G open the Move Atoms tool**, where you can move specific parts of your entry around if desired.
10. **The measure tool** found in the toolbar, allows you to select two atoms and measure the distance between them.

## C. CPU/GPU USAGE

All jobs that you run in BioLuminate will be run either on the CPU or the GPU. Small jobs, such as solvating a system, will be run on the CPU, whereas bigger jobs, such as running a molecular dynamic simulation, will be run on the GPU. Do not try to run MD simulations on the CPU, it will take too long and is too large of a job for the CPU to handle.

### ▪ CPU vs GPU

The CPU has an unlimited number of jobs allowed to be running at one time as long as the RAM is not overloaded. The number of jobs running on the GPU at one time is limited to the number of tokens we have purchased with our license. **Currently we can only run two MD simulations at one time.** If you start a third simulation, it will not be allowed, and the process will be killed. It is important to note that the chemical speed of the simulation (i.e. how fast it can be completed) will vary depending on if there is only one job running on the GPU vs two jobs running.

### ▪ CHECKING JOB STATUS

1. Click on the **Jobs** menu in the upper right of the BioLuminate window.

2. Click on **Monitor...**

a. This will open up the monitor where all jobs can be seen, and their status can be checked.

b. You can choose to show all active jobs, jobs from your project, all jobs, etc.

c. Looking at all active jobs may be helpful in deciding whether you can start a job on the GPU or not.

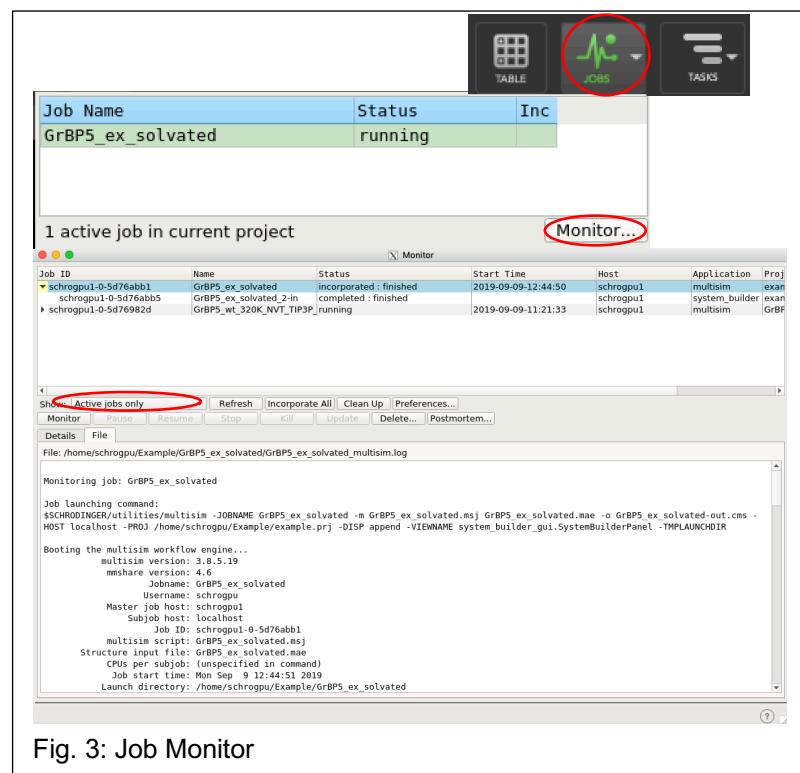


Fig. 3: Job Monitor

## D. LEAVING THE WORKSTATION

When you are finished with the workstation for the day, you may close out of open tabs including Maestro Bioluminate (it will ask you if you are okay quitting, and you usually are!). If there are any jobs running (yours or someone else's), **DO NOT close out of the terminals or the license configuration window**, or else the jobs in progress will be killed. Turn off the computer monitors by clicking the power button on the bottom right of the monitors.

## **II. BASICS OF USING DESMOND'S MD BIO LUMINATE SUITE**

*This section assumes that you have read the previous introduction to using the Schrodinger Workstation and know the basics of opening up a project, setting your working directory, and understanding how you can use the CPU and GPU to run your jobs.*

### A. BUILDING A PEPTIDE

1. In your project, click on the **Build** icon in the tool bar.
2. Go to **Other Edits > Build Peptide from Sequence...**
3. Enter a title for your peptide.
  - a. Note: use underscores instead of spaces in all of your naming in BioLuminate!
4. Enter the sequence and choose the **extended** structure shape.
5. Click **build**.

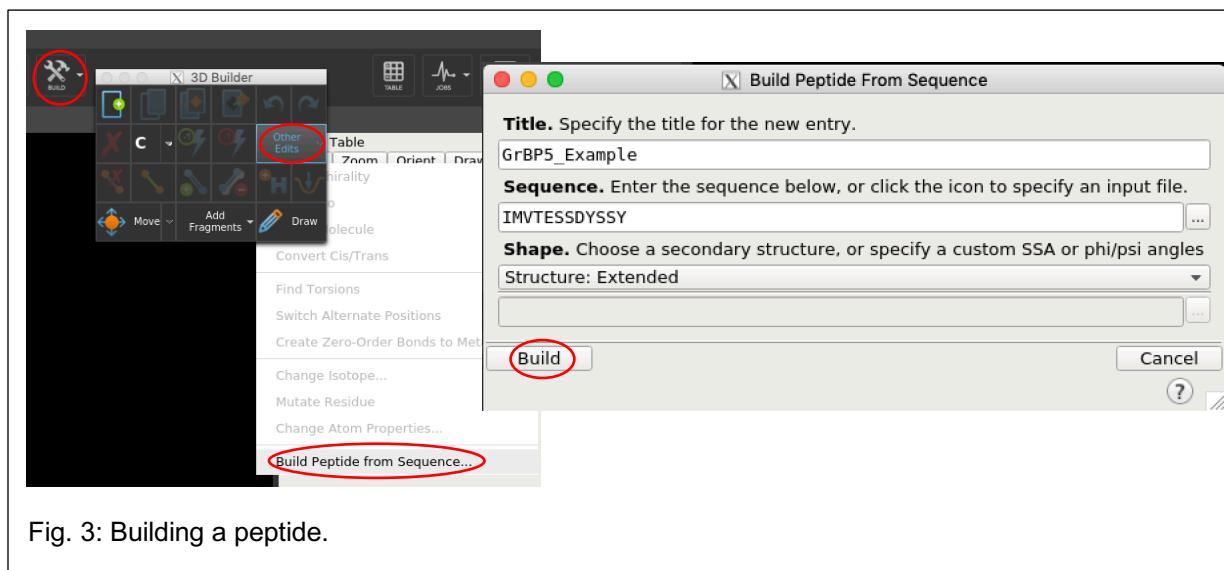


Fig. 3: Building a peptide.

6. Your peptide will be built and added as an entry in your entry list on the left of the BioLuminate window.
7. Fix the charges on your N and C termini following the steps found in *Altering Peptide pH*
  - a. The peptide is automatically built with N-terminus NH<sub>2</sub> and C-terminus COOH – this is not correct in nature, so we need to fix it!)
8. Highlight your newly built peptide and click on the “Protein Preparation” tab in the tool bar in BioLuminate.
9. Keep the default settings and click **Preprocess**.
  - a. This preprocessing step applies the force fields to your peptide so that you can work with it in BioLuminate.
10. You can change the style of your entry by clicking the **plus sign** next to the entry in the **Structure Hierarchy** panel.

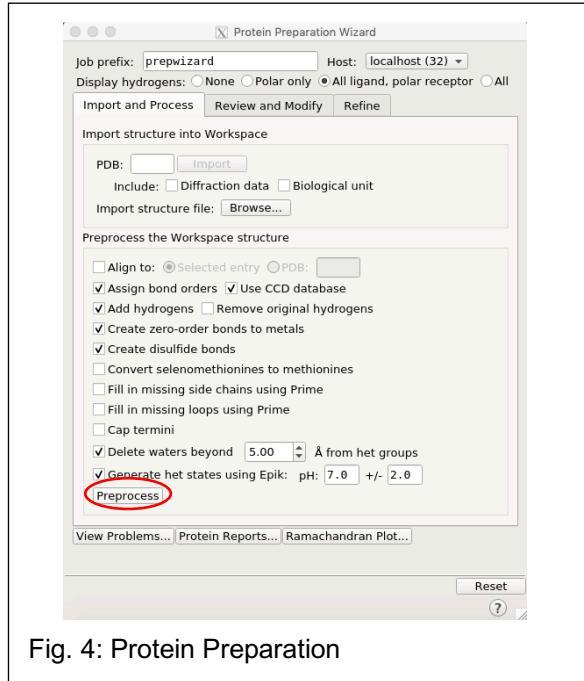


Fig. 4: Protein Preparation

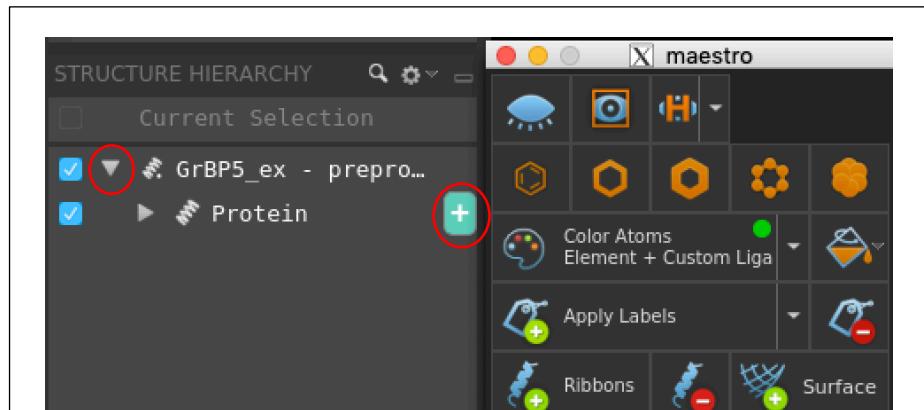


Fig. 5: Changing the entry style.

## B. SOLVATING YOUR SYSTEM

1. Click on the **Jobs** menu in the upper right of the BioLuminate window.
2. Click on “**System Builder**” in the list of recently visited panels or search for it.
3. In the **Solvation tab** in System Builder:
  - a. Choose your solvent (water) molecule. We typically use the predefined **TIP3P model**.
  - b. Choose your boundary conditions for your simulation box and **minimize the volume**.
    - i. You can use Buffer or Absolute size to create a boundary box. Buffer is easier as it will just create a buffer around your entry.
    - ii. Make sure your peptide has enough space to move around (10-15 angstroms buffer is usually sufficient).
4. In the **Ions tab**:
  - a. For ion placement, select **None**.
5. Name your job and click **Run**.
6. You can check the job status in the monitor (**Jobs > Monitor**).
7. Once completed, the solvated system will be incorporated into your entry list.

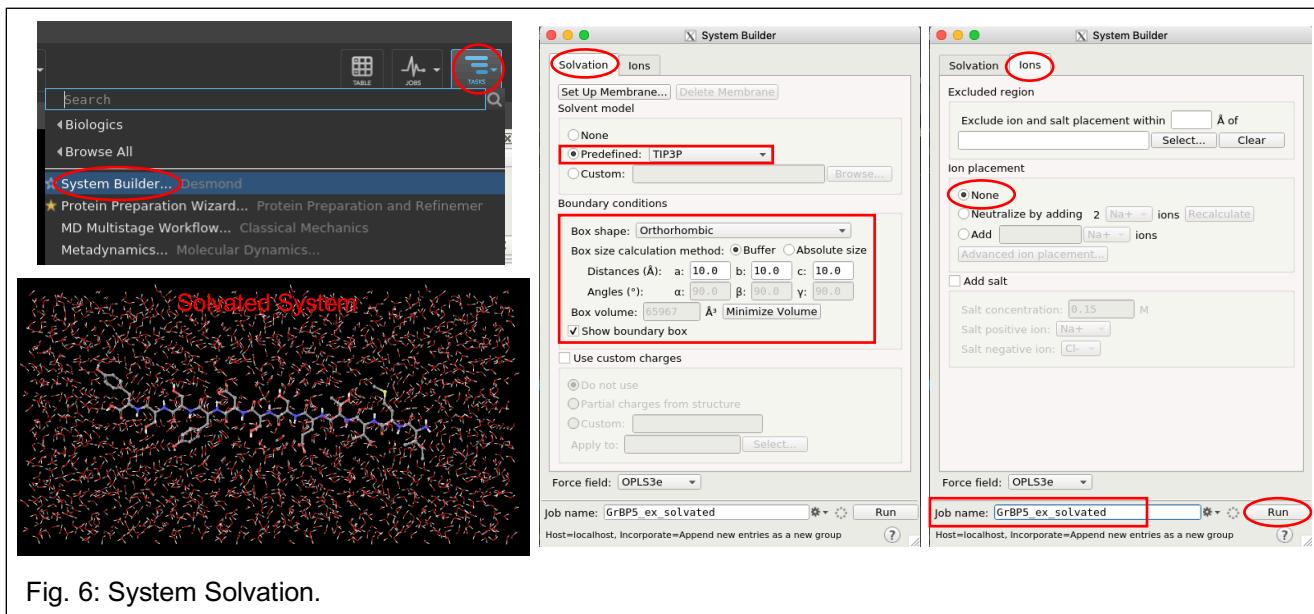
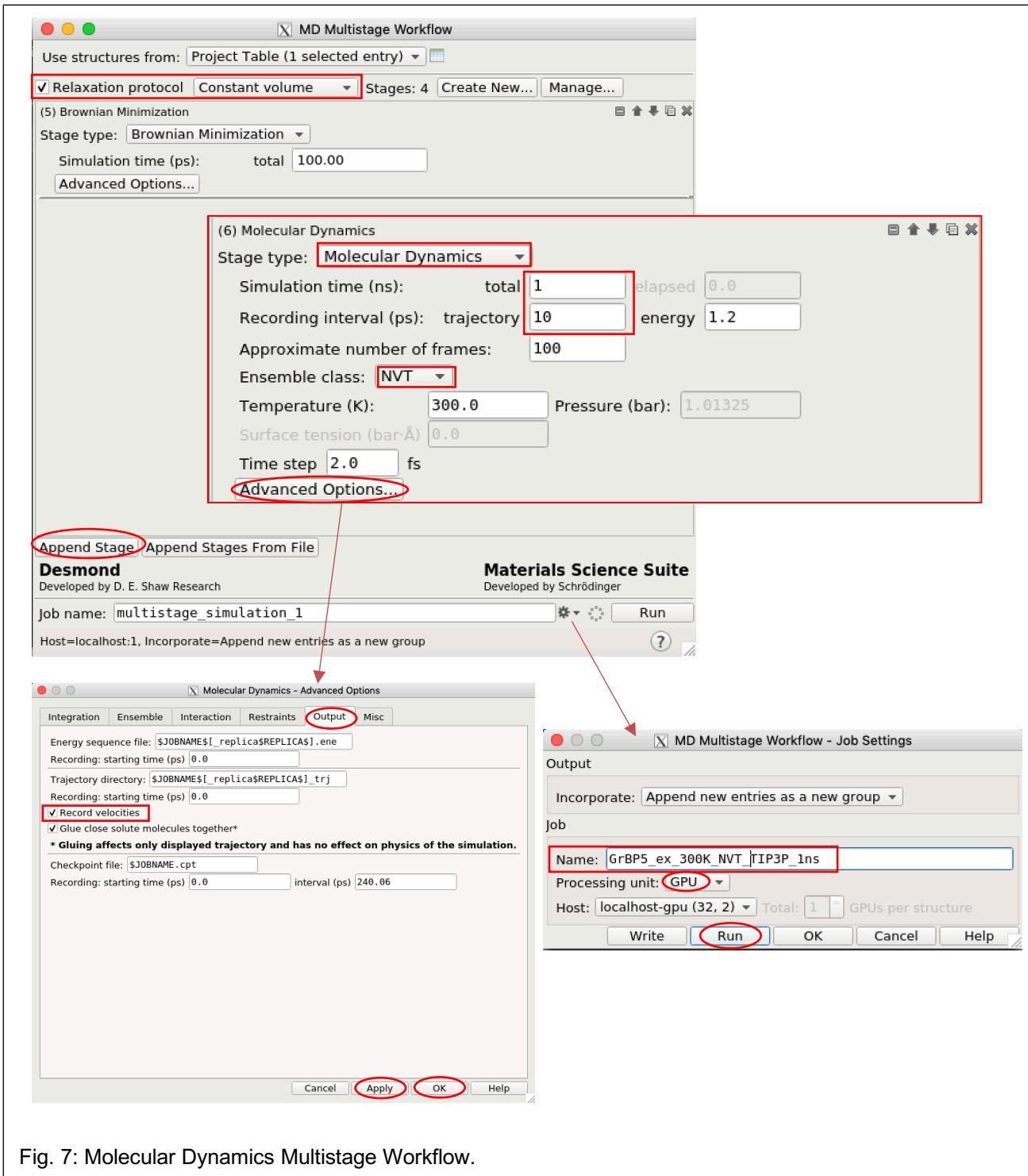


Fig. 6: System Solvation.

## C. STARTING A MOLECULAR DYNAMICS SIMULATION

Refer to Fig. 7 on page 11.

1. Highlight and put your solvated system entry into your workspace.
2. Click on the **Tasks** menu and open the “**MD Multistage Workflow**” panel.
3. Choose to use structures from “**Project Table (1 Selected entry)**”.
4. **Check the relaxation protocol** box and choose the **Constant volume** option.
5. Leave the Brownian Minimization on its default settings.
6. Click “**Append Stage**” at the bottom of the panel, this will add another stage in your workflow.
7. Change the stage type in the new stage to “**Molecular Dynamics**”.
8. Choose your simulation time.
  - a. It is important to run it for long enough to get accurate results and sample the conformational space of the peptide. Please look at the *An Example Project Protocol* section for more details.
9. Choose your recording interval.
  - a. I usually use a 10ps recording interval, but this depends on how much data you want from your simulation.
10. Select the ensemble class **NVT** (which keeps constant moles, volume, and temperature).
  - a. There are other ensemble classes you can use, but NVT is the most commonly used and has been found to be the best in most of our cases.
11. Change temperature as desired (default is 300K).
12. Click “**Advanced Options**”.
13. In the Advanced Options panel, go to the “**Output**” tab and **check Record Velocities**.
14. Click **Apply** and **OK**.
15. In the bottom right of the MD Multistage Workflow panel, click on the **gear > Job settings**.
16. Name your job and make sure it is running on the **GPU**.
17. Click **Run**. You can check progress in the monitor (Jobs > Monitor)



## D. EXTRACTING RESULTS FROM YOUR TRAJECTORY

Once your MD simulation is incorporated into your project, you can double click on the blue “T” next to your simulation in the entry list of your project to gain access to the **trajectory**. From here you can play the video, look at specific frames in the simulation, export data, and adjust playback settings. It may be helpful to hide the water molecules in your system to focus on your entry’s movement through the simulation. You can hide the water molecules by unchecking “Solvents” in the **Structural Hierarchy** panel in the bottom left of BioLuminate.

### ▪ VIDEO AND IMAGES

*To save images of your workspace:*

1. Set the style of your workspace and rotate your entry as desired.
2. In the **trajectory** panel click **Export > Image...**
3. Select where you wish to save the image and name it.
4. Another way to save an image:
  - a. At top of BioLuminate, click **Workspace > Save Image As...**

*To save a video of your trajectory:*

1. In the **trajectory** panel click **Export > Movie...**
2. Choose your desired frames, All in Range or a Limit range.
3. Choose your resolution, quality, and speed.
4. Click **Export**, name and save it.

## ■ MAE/PDB FILES

Schrodinger only allows single frames to be exported as a .PDB file. If you wish to have a .PDB file of the whole trajectory, you must first export as a .MAE file and then convert to a .PDB file.

*Exporting a .MAE file from an MD simulation:*

1. Hide your water molecules by unchecking solvents in the **Structural Hierarchy** panel and make sure all other atoms (that you want to be included in your .MAE file) are included in the workspace.

  - a. It's **very important** you are not including the water molecules in your .MAE file unless it's essential to your project. The water molecules will increase your file size exponentially and make things much harder for you.

2. Click **Export > Structures...**
3. Choose to export to File and Browse, name, and save.
  - a. Exporting to the Project Table will add it as a new entry to your entry list.
4. Select All in Range frames if you want the entire trajectory.
5. Choose Structures from Specified atoms.
6. Click + and choose “**displayed\_atoms**”.
  - a. Again, **do not** have your water molecules displayed!
7. Click **Export**.

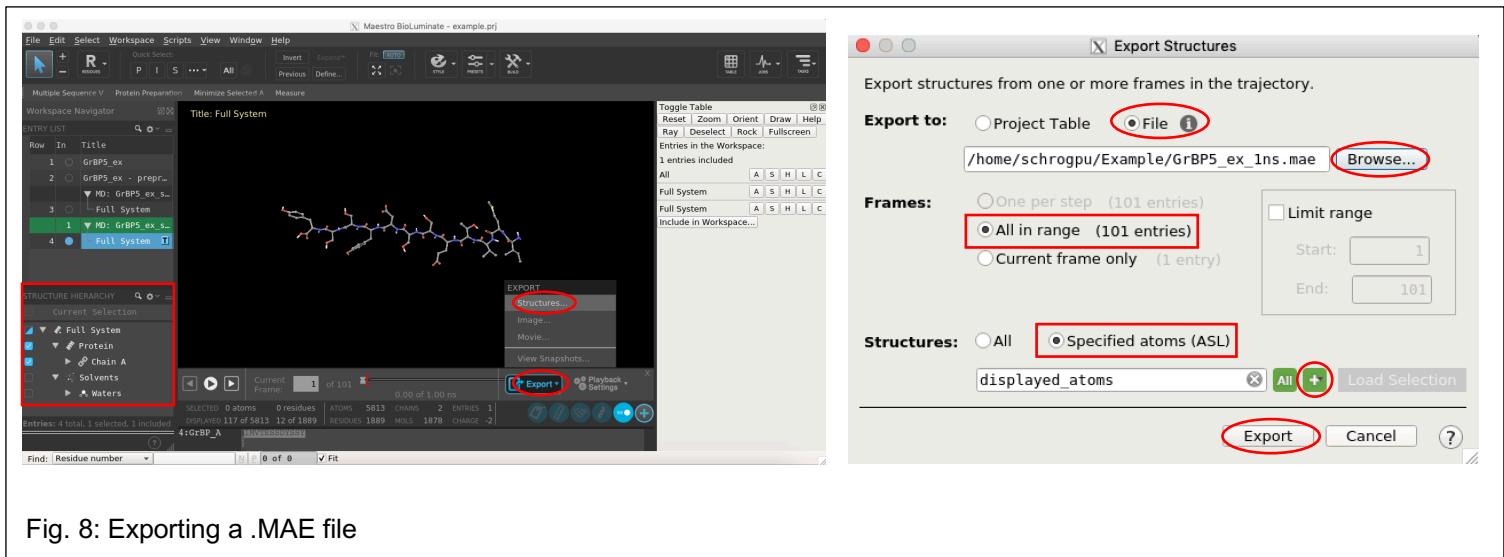


Fig. 8: Exporting a .MAE file

## Converting .MAE file to .PDB file

1. Right click on the desktop and open a new terminal.
2. Type “`export SCHRODINGER=/opt/schrodinger/2019-2`” and enter.
3. Go to the location where you saved the .MAE file.
  - a. Type “`cd.`” + where you saved the file. The default directory is Home, so if your file is in the Home folder you can type “`cd Your_folder/`”.
  - b. If your folder is elsewhere, for example on a hard disk, you will need to go to the correct location on the computer. Use “`cd`” to change your directory. If you type “`ls -l`” a list of files in your current directory will be printed, which may be helpful if you are trying to find your saved .MAE file.
4. Once you are in the correct directory, type “`$SCHRODINGER/utilities/pdbconvert -imae your_filename.mae -opdb your_filename.pdb`”.
5. This will create a .PDB file in the same folder where you saved your .MAE file.

```
(base) [schrogpu@schrogpu1 ~]$ export SCHRODINGER=/opt/schrodinger/2019-2
(base) [schrogpu@schrogpu1 ~]$ cd Example/
(base) [schrogpu@schrogpu1 Example]$ $SCHRODINGER/utilities/pdbconvert -imae GrBP5_ex_1ns.mae -opdb GrBP5_ex_1ns.pdb
```

Fig. 9: Terminal commands for MAE to PDB conversion.

### III. CREATING MORE COMPLEX SYSTEMS

#### A. GRAPHENE SIMULATIONS

##### ▪ BUILDING A GRAPHENE SHEET

1. Click on the **Tasks** menu in the upper right of BioLuminate.
2. Go to the “**Nanostructures...**” panel in the recently used tasks or search for it.
3. Change the fragment type to “**None**”.
4. Choose the size of the graphene sheet.
  - a. If you are running a graphene simulation with a peptide, its important that the graphene sheet is large enough to prevent the peptide from interacting with itself. For a 12 AA long peptide, I use a graphene sheet of 15x25 unit cells.
5. Name the job (usually its helpful to include the size you chose) and click **Run**.

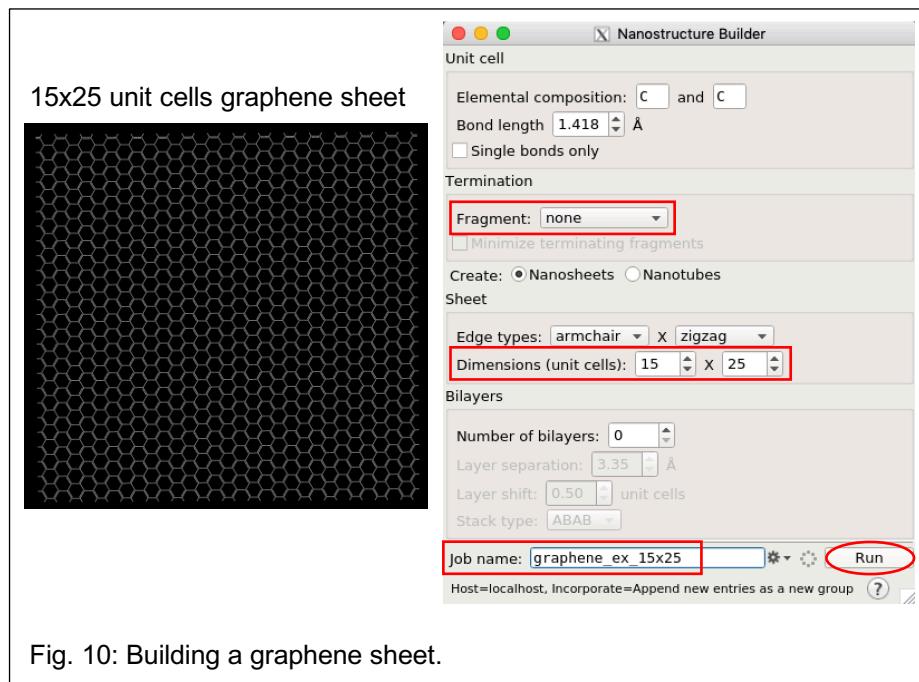
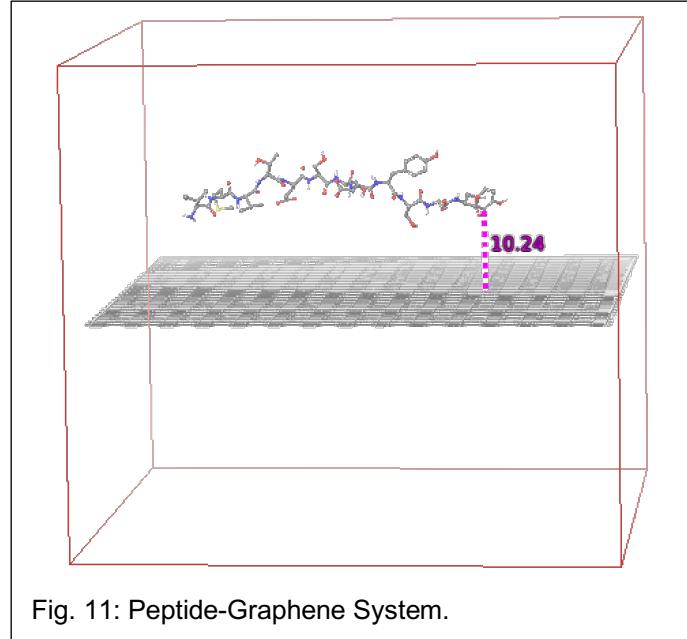


Fig. 10: Building a graphene sheet.

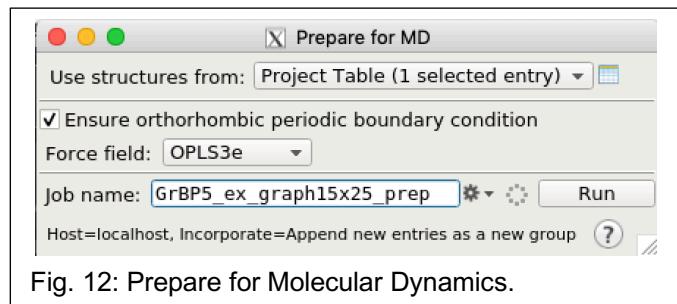
- **BUILDING A PEPTIDE-GRAPHENE SYSTEM**

1. Include both the peptide structure and the graphene in your workspace.
  - a. It is best to start with a water structure of the peptide above the graphene sheet in order to have a more accurate model (i.e. run water simulation first, then find the best structure to use in the graphene simulation).
2. Select the **entire peptide**.
3. Use the move tool (**Ctrl + Space + G**) to position and orient the peptide above the graphene sheet.
  - a. You can also use the **measure tool** in the toolbar to measure the distance between the graphene sheet and your peptide.
4. Go to **Tasks > System Builder**.
5. Change the settings as described in the previous section *Solvating your System*.
6. For the size of your simulation box:
  - a. **Start with a box 10x10x10 unit cells and minimize the volume.**
  - b. **Then change the dimensions** so that the box is tight against the graphene sheet and gives the peptide enough room to move in the z direction.
    - i. For example, for a 12 AA long peptide, 10 Angstroms above a graphene sheet, I may make my box 1x1x20 unit cells.
  - c. **Do not** minimize the volume again, this may cause the graphene sheet to bend. But don't worry, if it happens, just reset the box to 10x10x10 and minimize the volume and the graphene sheet should reshape itself correctly.
7. Name the job and run it.

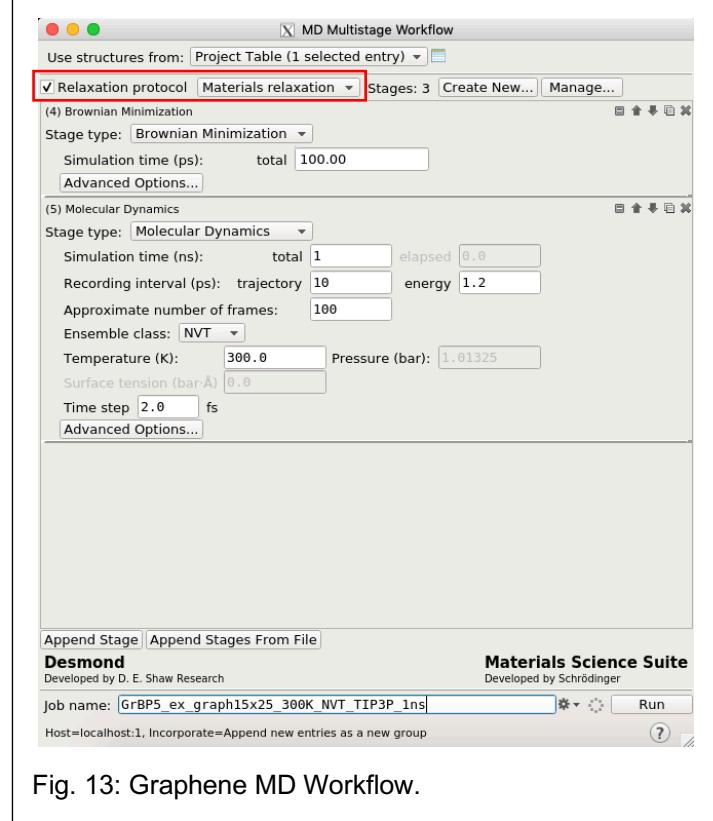


- **MD WITH GRAPHENE**

1. Include your solvated graphene simulation in the workspace and make sure it is highlighted.
2. Go to [Tasks > Prepare for Molecular Dynamics...](#)
  - a. This step will ensure that the periodic boundary conditions will remain in your system. **Do not skip this step**, your graphene sheet may become unbound and turn into a line rather than a sheet.
3. Keep the default settings:
  - a. Use structures from your Project Table.
  - b. Ensure orthorhombic periodic boundary conditions and OPLS3e force field.



4. Name your job and run it.
5. Once your prepped system is completed, view it in your workspace.
6. Go to [Tasks > MD Multistage Workflow](#).
7. Change the settings as described in the previous section *Starting a Molecular Dynamics Simulation*.
  - a. **Except make the following change:** For the relaxation protocol, use “Materials Relaxation”.
8. Name and run your job on the GPU.



## B. ADDING IONS AND CHANGING CONCENTRATION

### ▪ BUILDING IONS

1. Click on the **build icon** in the toolbar of BioLuminate.
2. Click on **the blue box with the green plus sign** to create a new entry in your entry list.
3. To create ions,

#### a. Using the Draw tool:

- i. Click on **Draw**.
- ii. Use the pencil tool to add atoms and bonds (Click on atom you want, then click in the workspace to add it).
- iii. Use the X tool to erase atoms and bonds.
- iv. Use the molecules already in the Draw Structure panel or click “...” to open the periodic table.

#### v. To add charge to your ion:

1. Open the **Protein Preparation** panel.
2. Go to the **Review and Modify** tab.
3. Click “**Analyze Workspace**”.
4. Highlight your ion in the Het list and click “**Generate States**”.
5. Check the **S2** box to classify the ion as charged.

#### b. Using the Add Fragments tool:

- i. Click on **Add Fragments**.
- ii. Add fragments as desired.
- iii. Click the “...” to see more options.

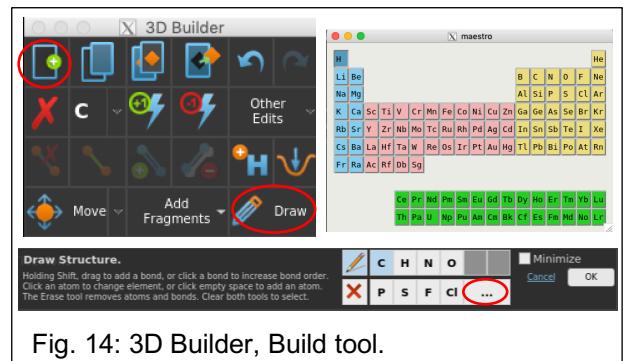


Fig. 14: 3D Builder, Build tool.

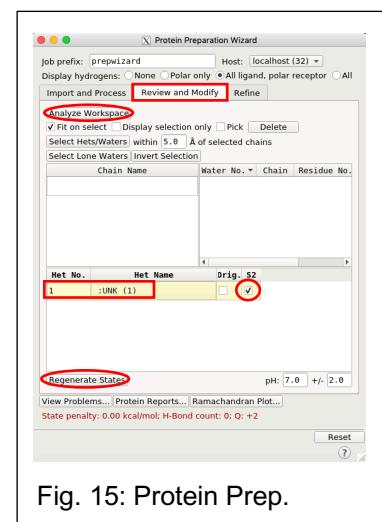
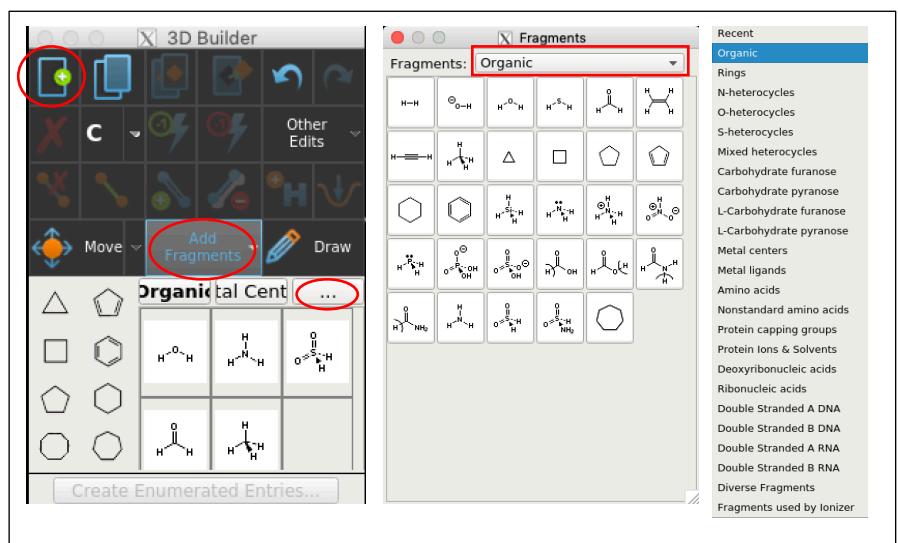


Fig. 15: Protein Prep.



## ■ CREATING A DISORDERED SYSTEM

1. Select all entries you want in your system (**they should be highlighted** – Ctrl + click on entries).
2. Go to **Tasks > Disordered System...**
3. Entry the **total number** of molecules your system will have.
4. Choose **how many of each entry** you want in your system.
5. Change the initial state to Amorphous.
6. Uncheck Steric Pack.
7. Click **Disordered Options:**
  - a. Change the water model to TIP3P.
  - b. If you wish to change the threshold distance of your system (how closely packed the molecules are):
    - i. Change the **initial density** (lower density = more spaced out molecules).
8. Click OK to close the Disordered Options panel.
9. Name your job and run it.

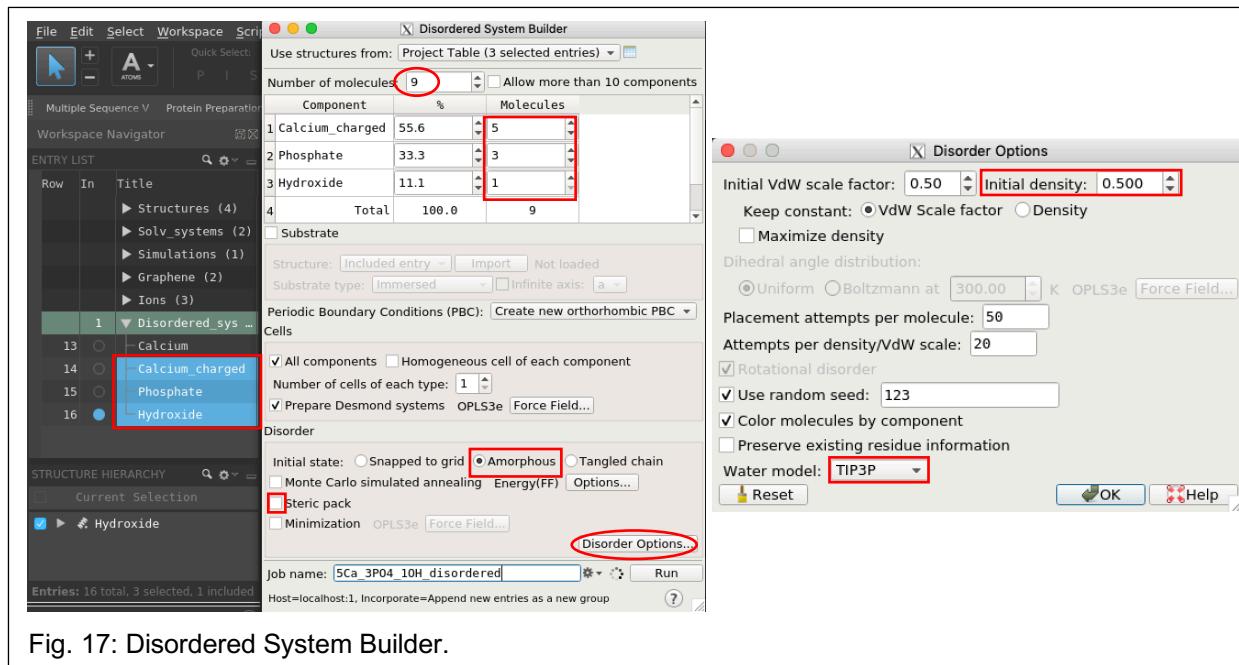


Fig. 17: Disordered System Builder.

## C. ALTERING PEPTIDE pH

Currently, in order to change the pH of a peptide, we have to **manually change the protonation states** of the amino acids. Please use an Amino Acid pKa chart to determine the charges of your amino acid sequence at a specific pH range. You may also use *PepDraw* (<http://pepdraw.com>) to aid you in determination of overall charge at a certain pH.

Once you have determined the desired protonation states of your peptide, follow these steps:

1. Go to the location on your peptide where you wish to add/remove hydrogens.
2. Change your **selection tool** to only **select atoms**.
3. **Select the atom** that you will be adding/removing hydrogens.
4. Click on the **Build** tool.
5. Click on the **lightning tool** (+1 to add H, -1 to remove H).
6. Continue this for all parts of the peptide chain you need to change
7. Make sure the **overall charge** displayed at the bottom of the BioLuminate screen is correct.

**Important Note:** when you first build a peptide in BioLuminate, the N-terminus is NH<sub>2</sub> and the C-terminus is COOH. This is not correct. At **neutral pH**, the **N-terminus should be NH<sub>3</sub><sup>+</sup>** and the **C-terminus should be COO<sup>-</sup>**. You will have to change this on your peptide.

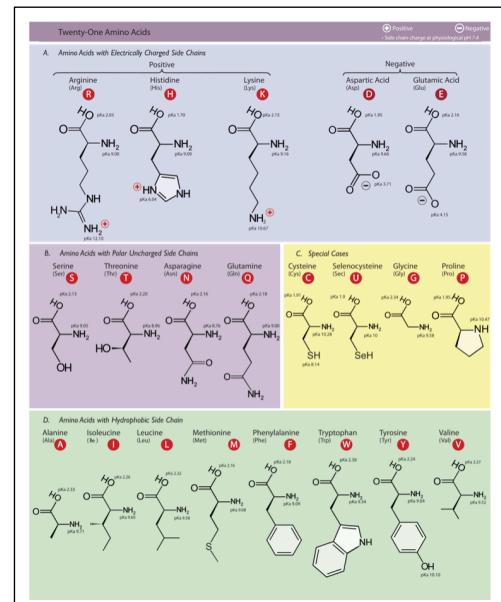


Fig. 18: Amino Acid pKa Chart.

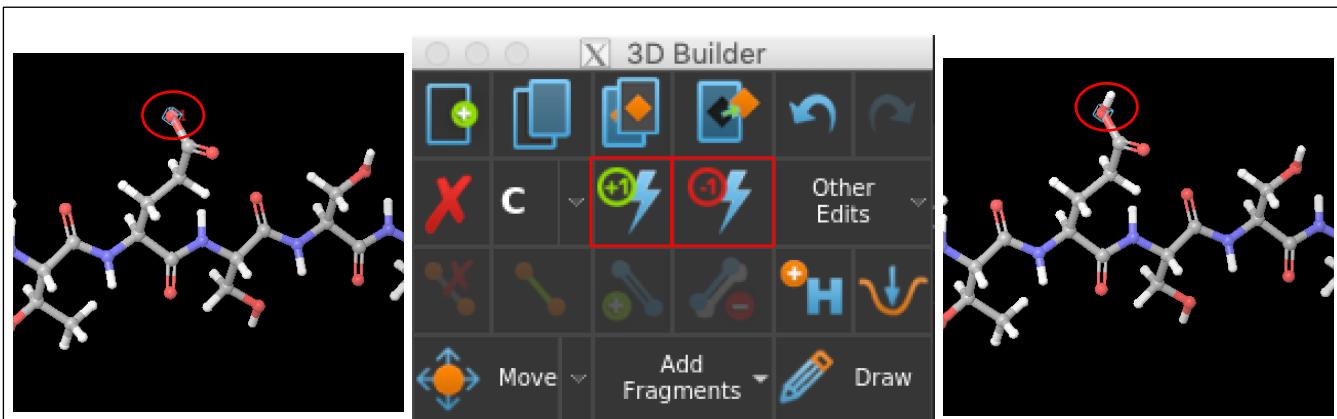


Fig. 19: Changing the protonation state of an amino acid.

## IV. OTHER TOOLS IN SCHRODINGER

Please note the following section includes some of the tools in Schrodinger, but this is not a complete list of all available tools.

### A. MACROMODEL CONFORMATIONAL SEARCH

1. Build an extended peptide and pre-process it, following the steps in [Building a Peptide](#).
2. Go to [Tasks > Conformational Search](#).
3. Include your peptide in your workspace and use the structures in your workspace.
4. Under the **Potential tab**, choose **water** as the solvent and leave the rest on default.
5. Under the **Mini tab**, change the method to “**SD**” and “**Converge on: Energy**”.
6. Under the **CSearch tab**, change the methods to “**Torsional sampling (MCMM)**”, uncheck the “**Retain mirror-image conformations**”, and use “**RMSD**” to eliminate redundant conformers.
7. Name and run your job.
8. Once your job is completed, it will incorporate different structures of your peptide.

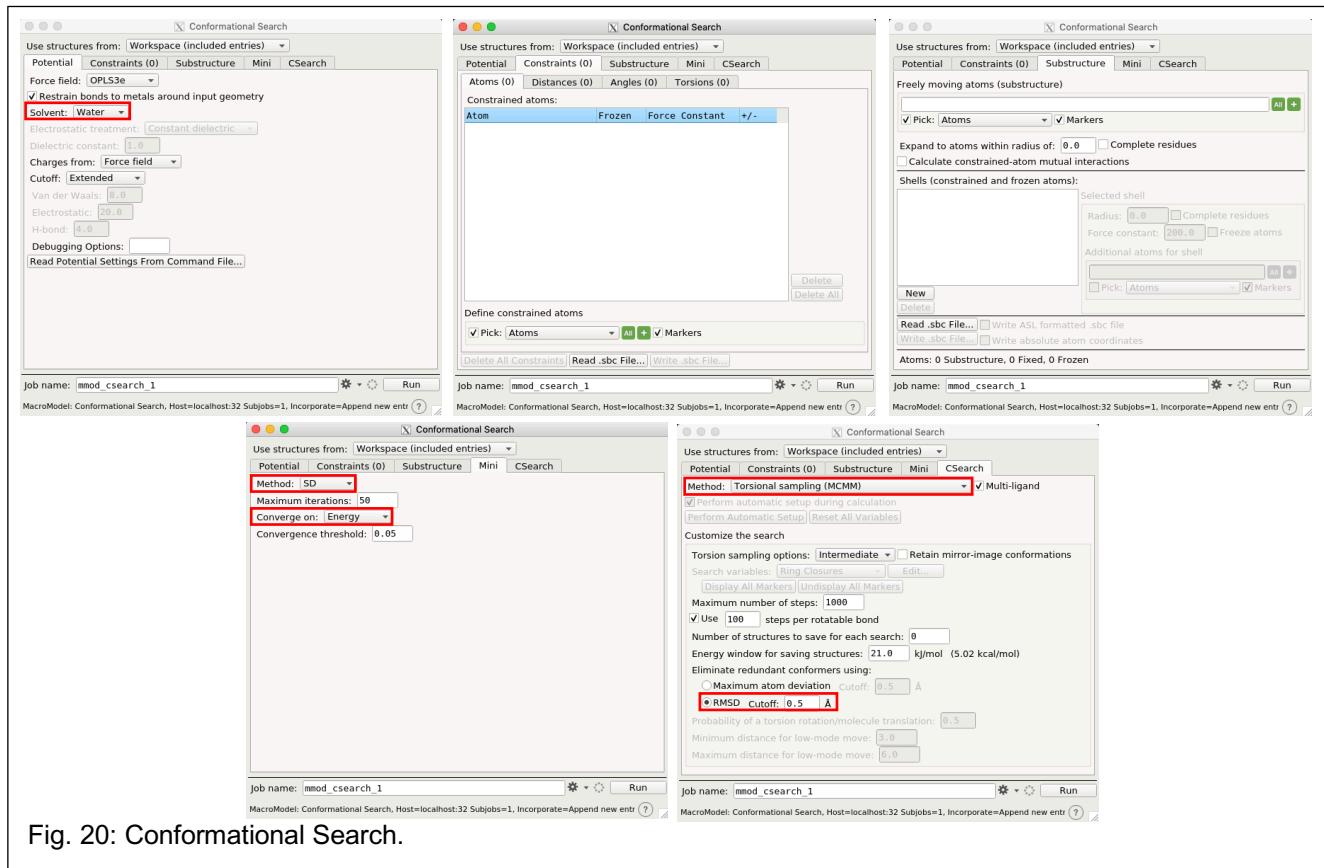


Fig. 20: Conformational Search.

## Clustering Conformational Search Structures

1. **Select all structures** generated from the conformational search.
2. **Tasks > Clustering of Ligands...** (Clustering Based on Volume Overlap panel).
3. Check “Normalized volume overlap”.
4. Click “Calculate Volume Overlap Matrix...”
5. Once the Volume Overlap Matrix is calculated, choose the Linkage Method: **Weighted Centroid**.
6. Click “Calculate Clustering”.
7. In the **Results tab**, you can view the results of your clustering.

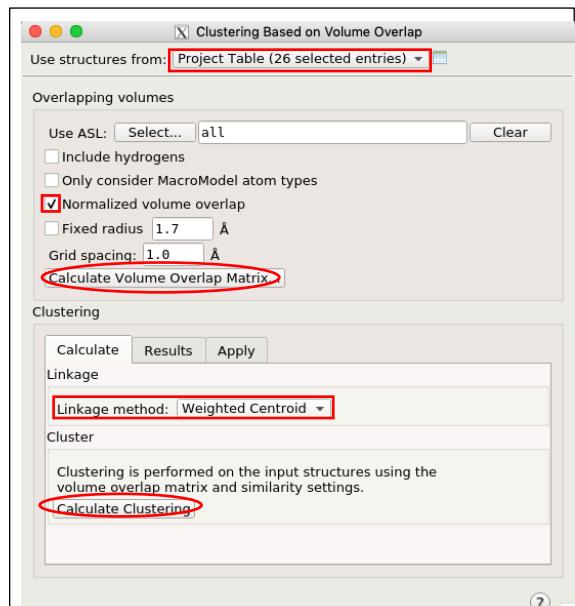


Fig. 21: Clustering Based on Volume Overlap

- a. **Note:** you can change the type of plot in the **Clustering Statistics** panel.
8. In the **Apply tab**, you can determine how many clusters you want and apply that to be displayed in your entry list.

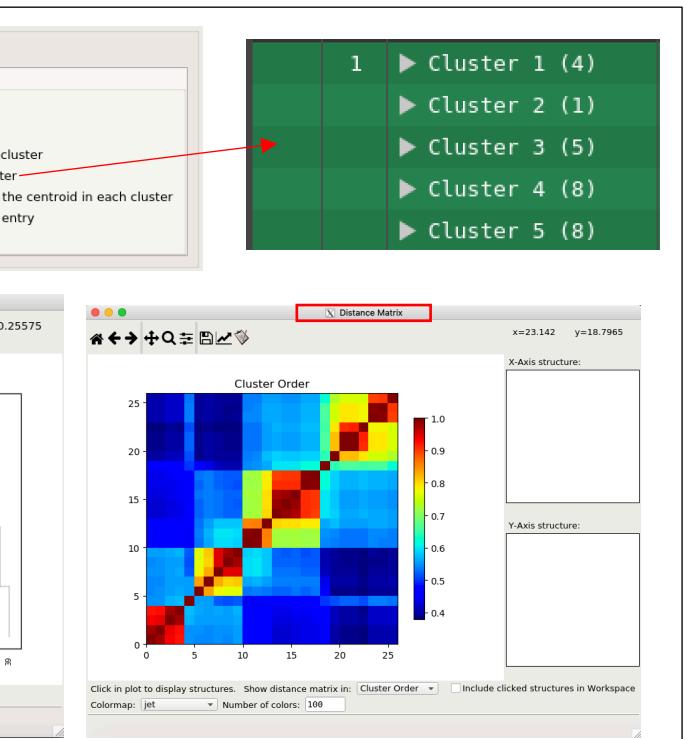


Fig. 22: Clustering Based on Volume Overlap Results.

## B. ENERGY TIME PLOTS

1. Tasks > Simulation Event Analysis.
2. Either Browse to find your trajectory or Import from Project Table.
3. In the Energy tab, select all properties you want to monitor.
4. Check “Pick molecule for energy properties” and click on the peptide in your workspace.
5. Once you select your peptide, a list of properties will pop up in the window.
  - a. You can choose what you want to analyze from this list.
  - b. We will look at the total energy of the system.
6. Select the properties you want, and click “Analyze”.
  - a. You can click “Analyze all” if you want to look at all the properties.
7. Enter a job name and click OK.
8. To view your results, select the properties and click “Analyze” in the Data Analysis section of the panel.
  - a. You can change the type of plot and how it is displayed.
9. You can also look at the different tabs in the Simulation Event Analysis Plot for more analysis. Please reference the Help page if interested in these analyses.

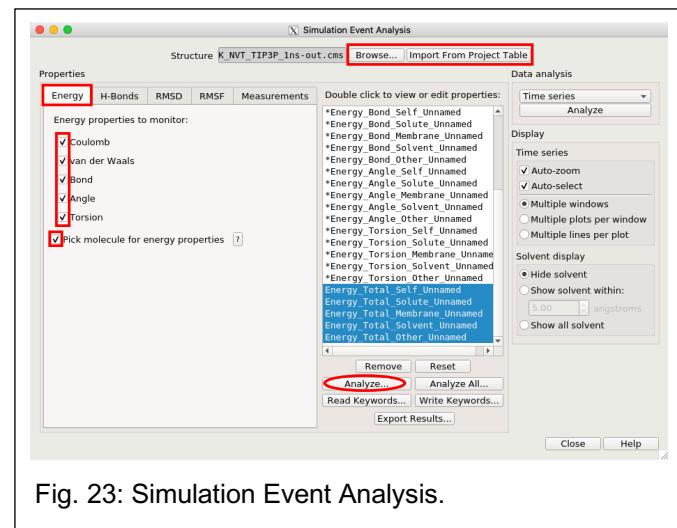


Fig. 23: Simulation Event Analysis.

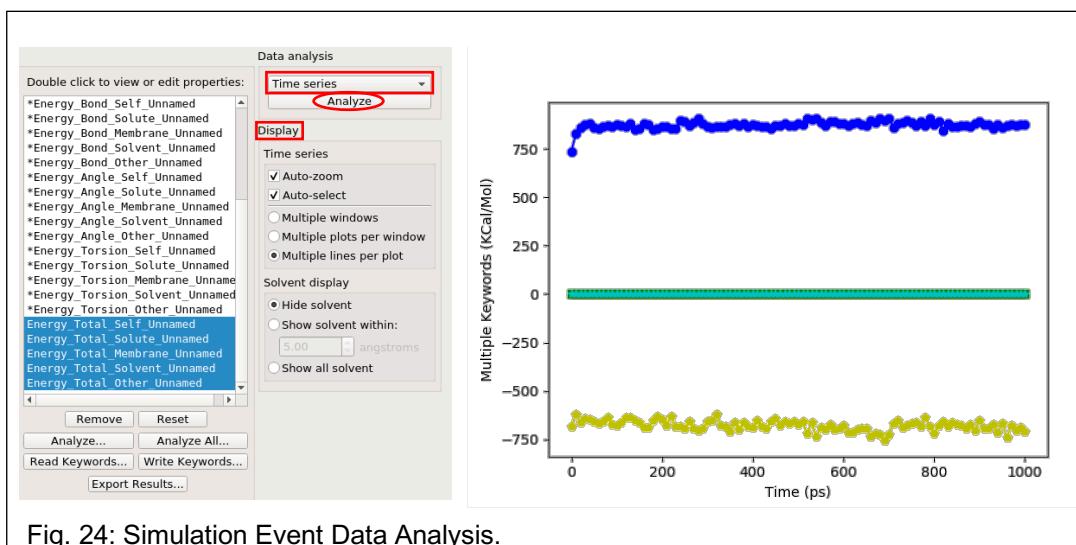


Fig. 24: Simulation Event Data Analysis.

## C. RADIAL DISTRIBUTION FUNCTION

1. Tasks > Radial Distribution Function.
2. Load your **-out.cms** file from your trajectory.
3. Choose to group your molecules by “**Center of Mass**”.
4. Select your atoms you want and click OK.
5. Name your job and run it.
6. In the **View Results tab**, you can save your results.

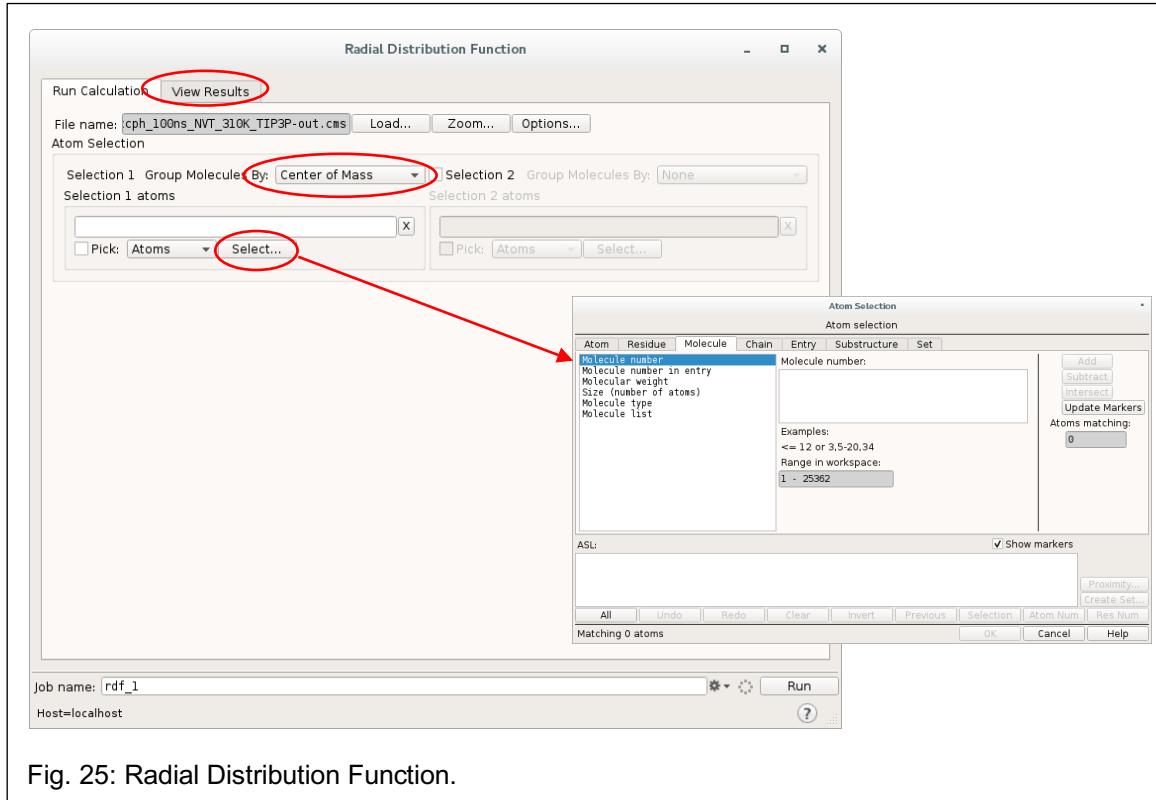


Fig. 25: Radial Distribution Function.

## D. ENHANCED SAMPLING TECHNIQUES

### ▪ REPLICA EXCHANGE

*Replica Exchange is an Enhanced Sampling Technique that replicates the original chemical system into  $N$  copies and each replica is independently propagated with a different Hamiltonian. States are exchanged depending on temperature and energy differences to sample the replica space.*

*REMD (replica exchange molecular dynamics) – parallel calculations are performed on the entire system at different temperatures.*

*REST (replica exchange with solute tempering) – potential energy is adjusted for just the critical part of the system (e.g. the peptide).*

Schrodinger's Replica Exchange task will produce one trajectory for each replica.

1. [Tasks > Replica Exchange](#).
2. Load in your system.
3. Choose your tempering method (REMD or REST).
4. Choose the number of replicas you want (default is two).
5. For REMD:

- a. Choose your temperature range.
- b. Choose the temperature profile (i.e. how the temperature will change with each replica).

6. For REST:

- a. Choose your low temperature.
- b. Select your Hot Region (i.e. the peptide).
- c. A high temperature will be selected for you.

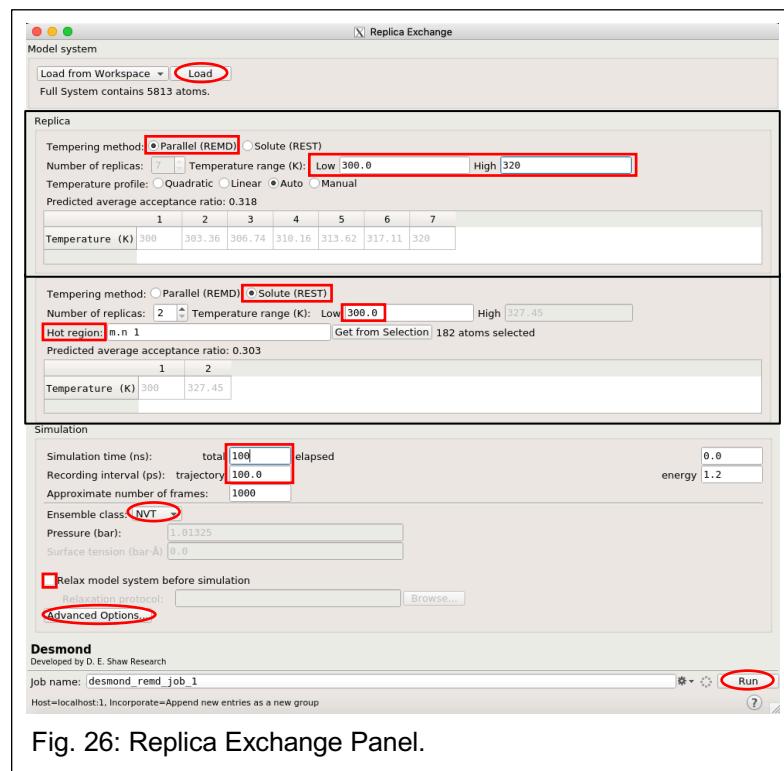


Fig. 26: Replica Exchange Panel.

7. Choose your simulation time and recording interval.
8. Choose NVT as your ensemble class.
9. If you have a relaxation protocol (.msj file), you may use it here. Otherwise you can first run Brownian minimization in the MD Multistage Workflow panel (as in classical MD simulations) then use the result of that job in the Replica Exchange.
  - a. This is needed since we are preparing our model systems with system builder.
10. In the **advanced options**, go to the **output tab** and check “**Record Velocities**”.
11. Name your job and run it.

To analyze the results of your Replica Exchange:

1. [Tasks > Replica Exchange Review](#).
2. Load your log file for the simulation.
3. Choose to look at one or all replicas and click “[Display Plot](#)”.
  - a. Here you can see how the replicas exchanged through the simulations.

## ■ METADYNAMICS

*Metadynamics is an Enhanced Sampling Technique in which the potential for chosen variable(s) is adjusted by periodic addition of repulsive Gaussians. The repulsive Gaussians will fill up the well being sampled and force the calculation into another well in order to sample more of the conformational space and to discourage sampling of the already-sampled space.*

*The height and width of the Gaussian and the interval in which the Gaussians are added control the accuracy of the simulation. A decrease in heights and an increase in the time interval will increase the accuracy, but also increase the time needed for the simulation to be completed. The width of the Gaussian should be ~1/4 to 1/3 of the average fluctuations of the collective variable during a classical MD simulation.*

1. [Tasks > Metadynamics](#).
2. Choose the type of variable (Distance, Angle, or Dihedral).
3. Select the atoms that define that variable.
4. Set the width of the Gaussian.
5. If variable is Distance:
  - a. You may choose to place a “wall” to prevent the system from moving too far in the direction defined by the variable.
6. Click “Add” to add the variable.
7. Choose your Relaxation protocol (.msj file) if you are relaxing the system before simulation.
8. Set your simulation settings.
9. Under **Advanced Options**, choose to Record Velocities on the output tab.
10. Name and run your simulation.

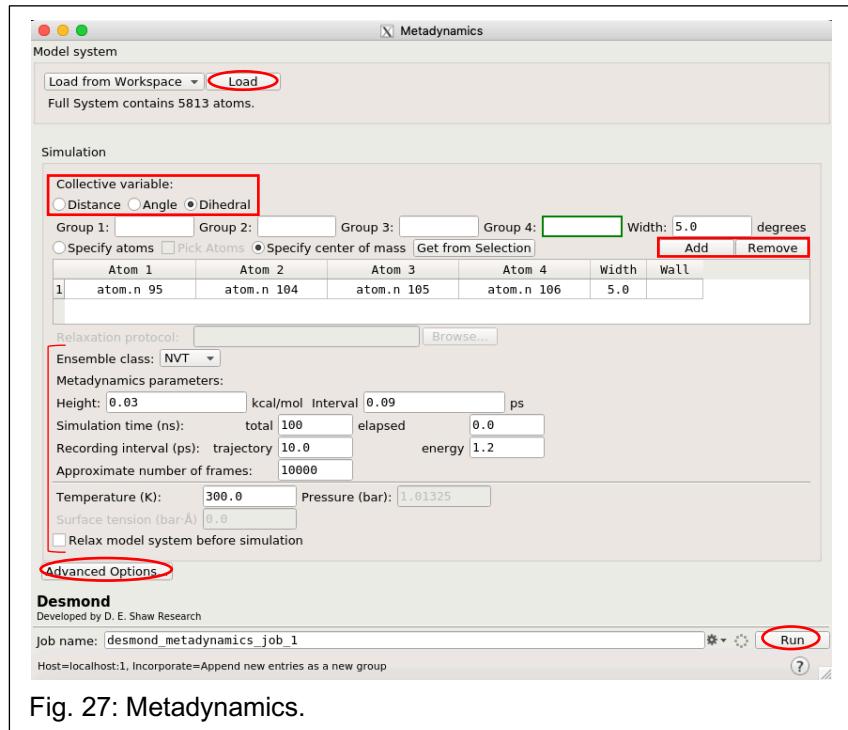


Fig. 27: Metadynamics.

## V. AN EXAMPLE PROJECT PROTOCOL

The following section includes an example protocol for an MD project.

### Determine a Water Structure of the Peptide

*It is important to first determine the water structure(s) of your peptide in order to create the most realistic systems. Replica exchange and Metadynamics can be used to sample the conformational space. Sometimes it is also necessary to get microseconds of MD data to sample the space and determine the most sampled conformations.*

For simple water structure determination:

1. Build a peptide.
2. Adjust the N and C termini to reflect pH 7.
3. Pre-process your peptide and create a solvated system.
4. Run a 200ns simulation to allow the peptide to fold.
5. If you are interested in changing pH:
  - a. Take the last frame or a commonly sampled frame from the 200ns simulation and export it into your project table.
  - b. Change the pH of the folded peptide.
  - c. Run 200ns simulation at the new pH.
6. Use the 200ns water structure in your more complex systems (with graphene, ions, etc.).

### Run your peptide water structure in your more complex systems

1. Use the water structure(s) to create your system with graphene, ions, etc.
2. Run your simulation(s) for 50-200ns depending on how much data you want.

### Analyze your trajectory

1. Analyze your trajectory with Schrodinger tools.
2. Export the mae/pdb file(s) from your simulation.
3. Analyze the pdb file in python, chimera, etc.
  - a. Hilbert PCA
  - b. Ramachandrans
  - c. Chimera is useful for analyzing and viewing your pdb (freely downloadable online)

## VI. TROUBLESHOOTING

The following section will help to solve some common problems in Schrodinger. Please ask for help if you need it!

### If your job died...

- Did you preprocess your peptide?
- If you are trying to run your job on the GPU, are there enough tokens? (i.e. are there 2 jobs already running on the GPU?)
- Did you exit out of the terminal running your job?

### If you are asked to Wait or Force Quit...

Always click **Wait!**

Schrodinger sometimes will take time to load things and you just have to wait. If you force quit, you may risk the chance of killing any jobs that were running.

### If you are having problems with your MD Trajectory...

If you are getting an error when you double click the trajectory T on a simulation... This problem may occur if you moved files around. When you run an MD trajectory the file saves in your working directory. If you forget to change your working directory to the correct folder and you move the files manually, this may affect your access to them.

To fix this, you can import the structures again by going to File > Import Structures. Go to the job that you want and import the -out.cms file.

If something has happened to your files where simply importing them again won't work, you can try to rescue the trajectory. To do that follow these steps:

1. Open a new terminal
2. Type “`export SCHRODINGER=/opt/schrodinger/2019-2`”
3. Go to the file where your trajectory is saved (use “`cd`” to change your working directory)
4. Type “`$SCHRODINGER/run trj_rescure.py yourfilename-out.cms yourfilename_trj -o fixed`”
5. This will create a fixed -out.cms file that you can import into your project
6. If this does not make sense, please ask for help!!