

Winmostar tutorial

Gromacs

Protein

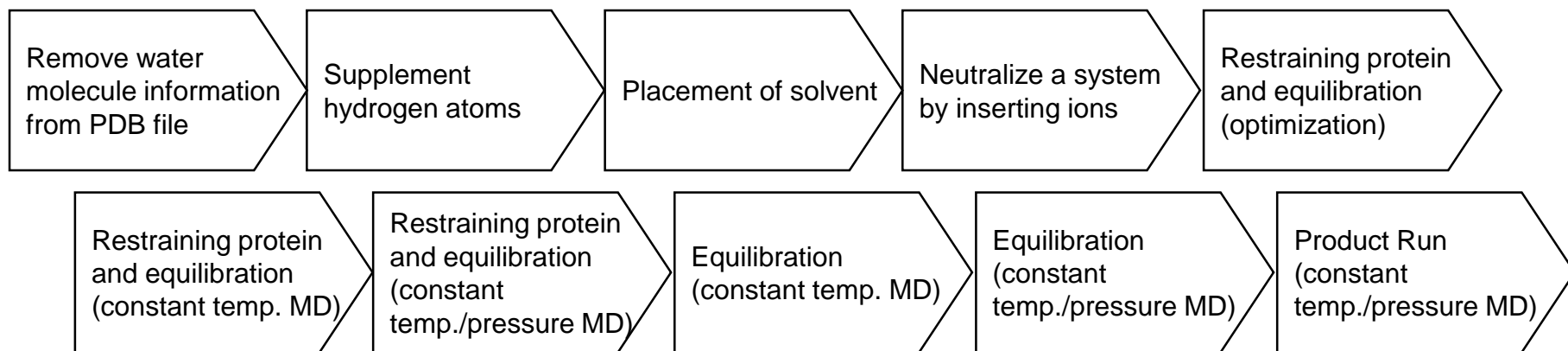
V8.007

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Summary

- In this tutorial, we will use Gromacs to execute calculations on a PDB file of Hen Egg-White (HEW) Lysozyme.



Notes:

- It is necessary to remove crystal water data contained in the XRD of PDB and supplement hydrogen which is not initially included.
- Size of systems (number of solvents) will affect behavior of proteins.
- The number of steps required for equilibration depends on the type of molecule and initial density varies and may be different from this example.
- The method for interaction calculations and/or the force field also affect simulation results.

Configuration

You must set up Cygwin to use Gromacs on Winmostar.

- Obtain the installer for Cygwin, which contains the all programs needed by Winmostar, at https://winmostar.com/en/manual_en.html.

2. Installation Guides for Solvers

For Windows

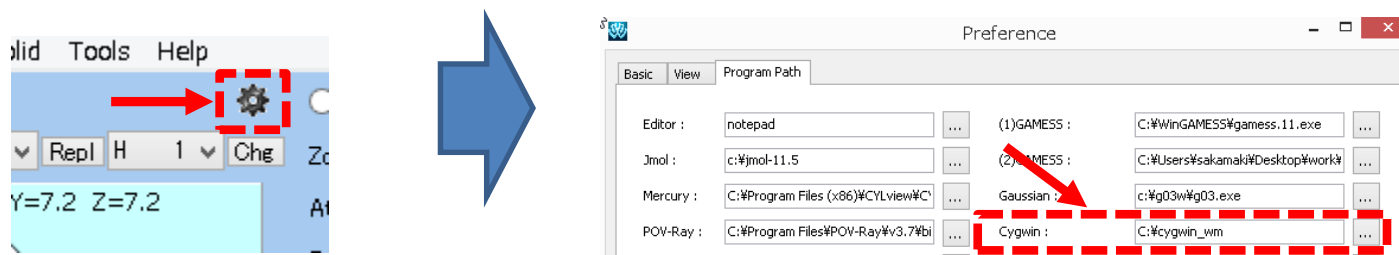
[cygwin_wm_v7_20160926.exe\(413MB\)](#) ※NWChem/Gromacs/Amber Window Build Package(Cygwin)

(For Experts)NWChem/Gromacs/Amber Build with Cygwin ※we recomend to use cygwin_wm_v7_20160926.exe

[GAMESS Installation Guide](#)

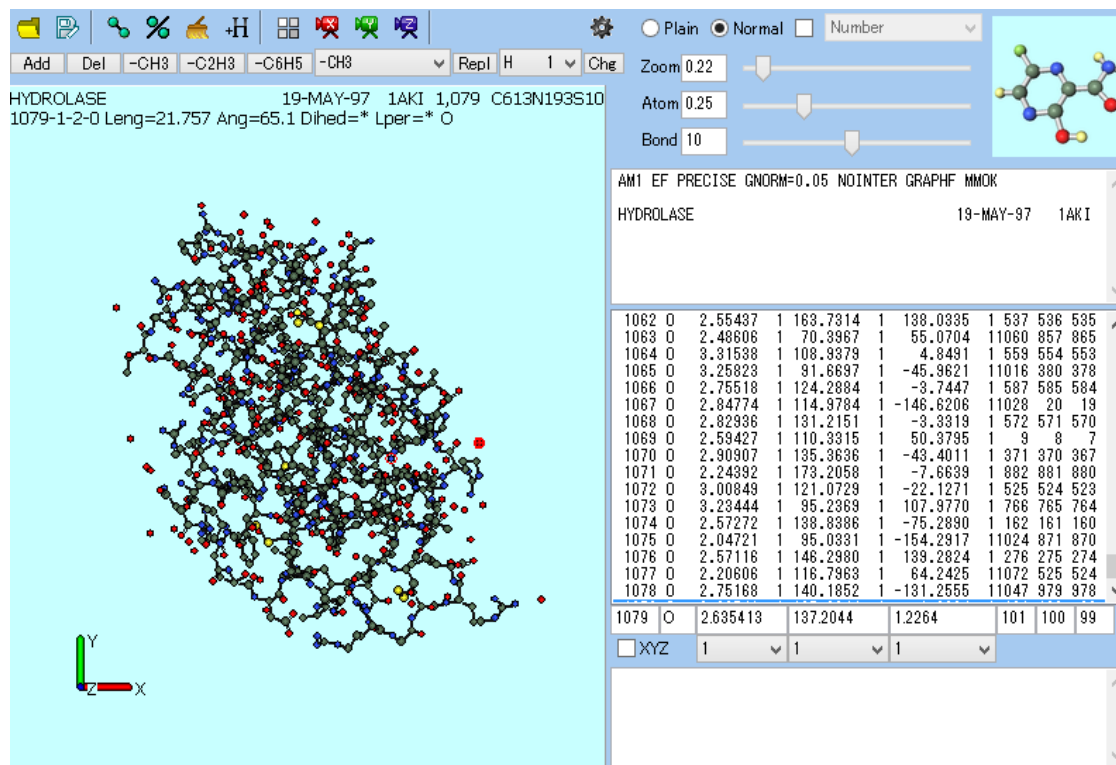
[LAMMPS Installation Guide](#)

- When you change the installation path for Cygwin from the default one, specify it on the preference panel.



I. Build a simulation cell

1. Click **File | Open**.
2. Open **1AKI.pdb** in sample directory.
(default: C:\winmos8\samples\1AKI.pdb)



I. Build a simulation cell

1. Click **Edit | Select Molecules.**
2. On **Select Molecules** window, check "**O 78.**" This will add blue circles around oxygen atoms of crystallization water as shown below (i.e. partial selection).

The screenshot displays the X-Ability software interface. On the left, the 'Edit' menu is open, with 'Select Molecules' highlighted. A yellow arrow points from this menu item to the 'Select Molecules' dialog box on the right. The dialog box shows a list of atoms with the entry 'O 78' checked. Another yellow arrow points to this entry. The main window shows a 3D ball-and-stick model of a protein-ligand complex. A blue arrow points from the 'Select Molecules' dialog to the 3D model, indicating the selection of atoms. The 3D model shows a protein backbone in grey, with various atoms colored by element (carbon in grey, oxygen in red, nitrogen in blue). A coordinate system (X, Y, Z) is visible at the bottom left of the 3D view.

Select Molecules dialog box content:

| Composition | # Mol |
|------------------------------------------|-------|
| <input type="checkbox"/> C613N193S10O185 | 1 |
| <input checked="" type="checkbox"/> O 78 | 78 |

Buttons: All, None, Invert, Update List

3D Model Text: HYDROLASE 19-MAY-97 1AKI 1,079 C613N193S10 1079-1-2-0 Leng=21.757 Ang=65.1 Dihed=* Lper=* O

Atom List (partial):

| | | | | | |
|--------|---------|----------|-----------|--------|---------|
| 1082 O | 2.55437 | | | | |
| 1083 O | 2.48606 | | | | |
| 1084 O | 3.31538 | | | | |
| 1085 O | 3.25828 | | | | |
| 1086 O | 2.75518 | | | | |
| 1087 O | 2.84774 | | | | |
| 1088 O | 2.82936 | 131.2151 | -3.3319 | 1.572 | 571 570 |
| 1089 O | 2.59427 | 110.3815 | -50.3795 | 1.9 | 8 7 |
| 1070 O | 2.30907 | 135.3636 | -43.4011 | 1.371 | 370 367 |
| 1071 O | 2.24392 | 173.2058 | -7.6639 | 1.882 | 881 880 |
| 1072 O | 3.00849 | 121.0729 | -22.1271 | 1.525 | 524 523 |
| 1073 O | 3.23444 | 95.2369 | 107.9770 | 1.766 | 765 764 |
| 1074 O | 2.57272 | 138.8386 | -75.2890 | 1.162 | 161 160 |
| 1075 O | 2.04721 | 95.0331 | -154.2917 | 1.1024 | 871 870 |
| 1076 O | 2.57116 | 146.2980 | 139.2824 | 1.276 | 275 274 |
| 1077 O | 2.20606 | 116.7963 | 64.2425 | 1.1072 | 525 524 |
| 1078 O | 2.75168 | 140.1852 | -131.2555 | 1.1047 | 979 978 |

Summary Table:

| | | | | | | |
|--------|----------|----------|--------|-----|-----|----|
| 1079 O | 2.635413 | 137.2044 | 1.2264 | 101 | 100 | 99 |
|--------|----------|----------|--------|-----|-----|----|

XYZ: 1 1 1

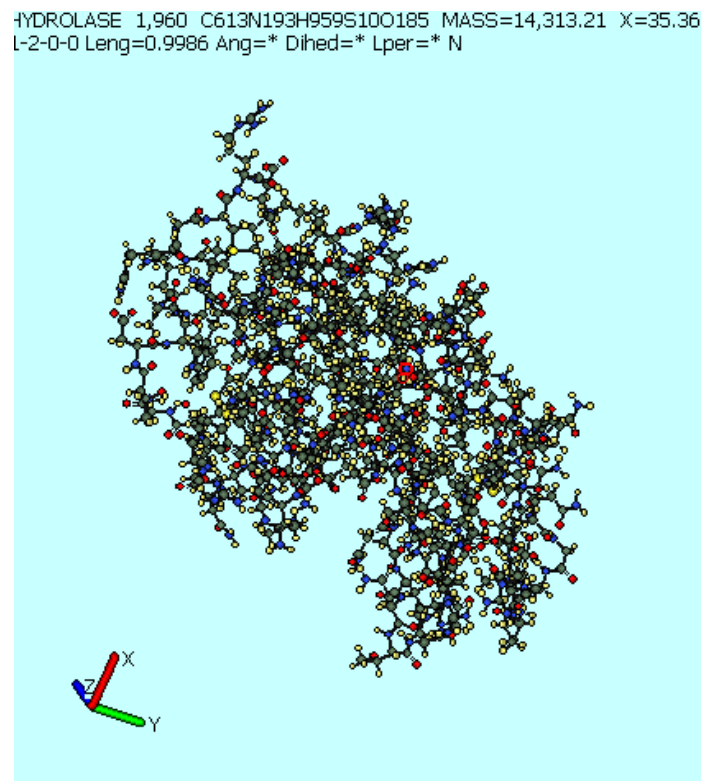
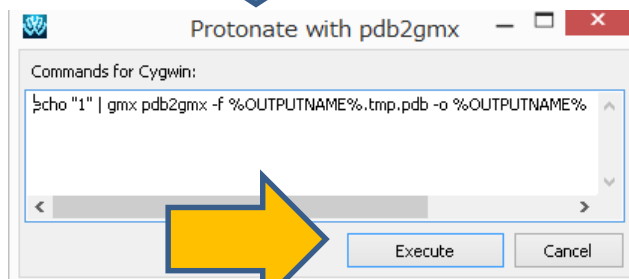
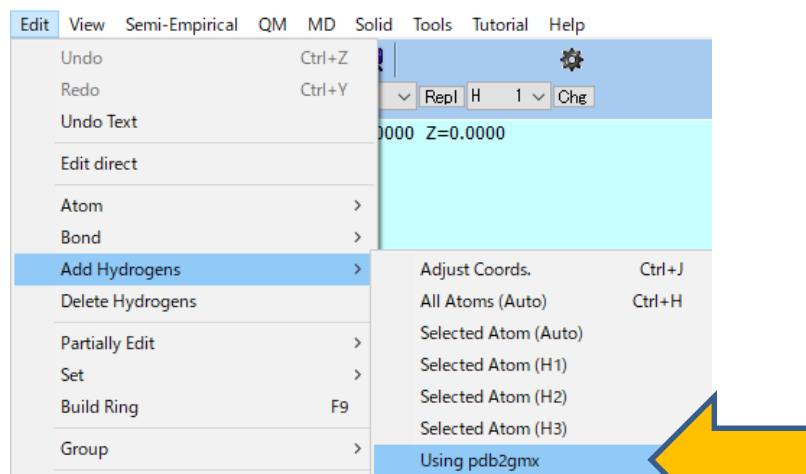
I. Build a simulation cell

1. After ensuring that oxygen atoms have been selected in the crystallization water, click **Edit | Partial Edit | Partial Delete**.
2. Click **Delete** on **Selection** to delete crystallization waters.

The screenshot illustrates the software interface for deleting crystallization waters. On the left, the 'Edit' menu is open, showing the path: **Edit** > **Partially Edit** > **Partially Delete (X)**. A yellow arrow points to this menu item. Below the menu, a blue arrow points to a 'Selection' dialog box. This dialog box contains a 'Delete or Leave?' prompt and three buttons: 'Delete', 'Leave', and 'Cancel'. A yellow arrow points to the 'Delete' button. To the right, a 3D molecular model of a protein (HYDROLASE) is shown in a light blue simulation cell. A coordinate system (X, Y, Z) is visible in the bottom left of the model view. Text at the top right of the model view reads: 'HYDROLASE 19-MAY-97 1AKI 1,001 C613N193S10 1-2-0-0 Leng=1.4812 Ang=* Dihed=* Lper=* N'.

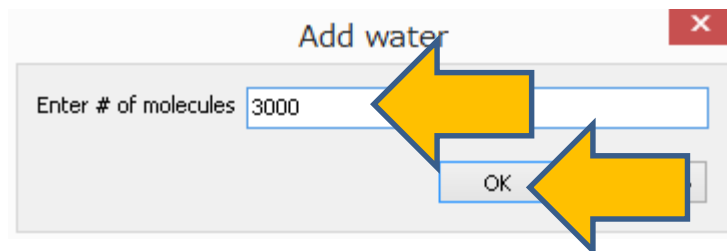
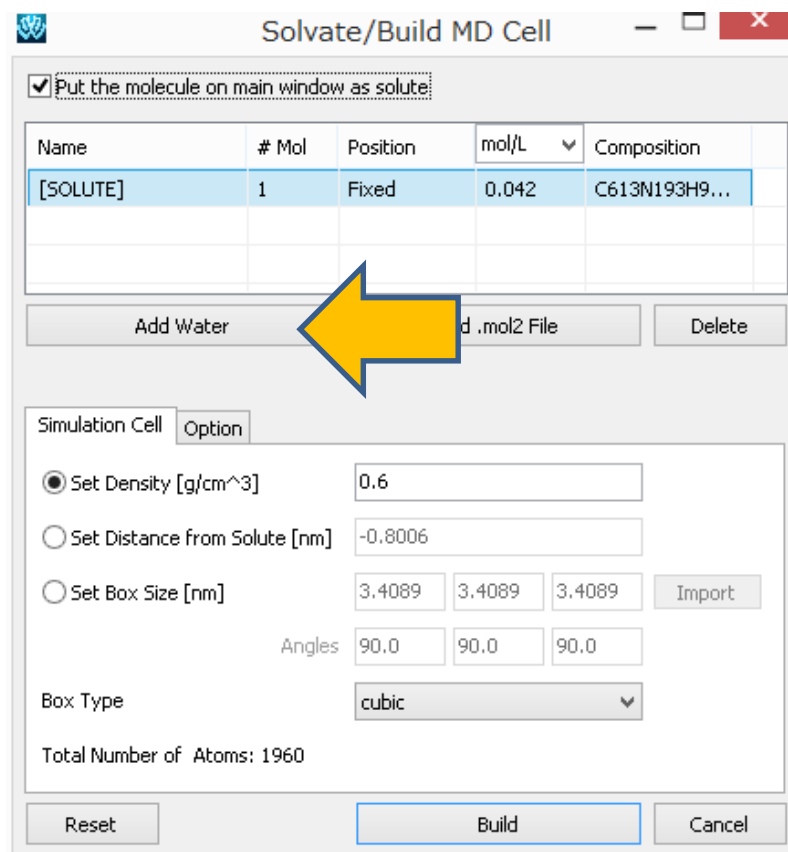
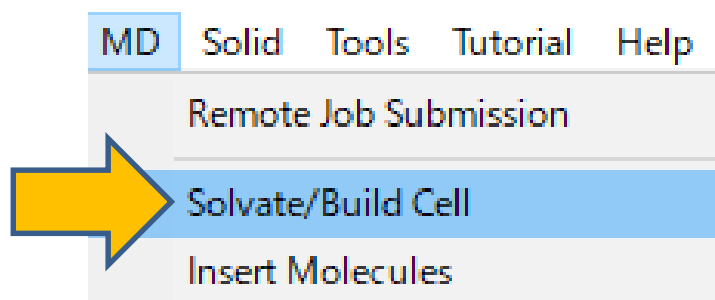
I. Build a simulation cell

1. Click **Edit | Add hydrogens | Using pdb2gmx.**
2. In the **Protonate with pdb2gmx** window, click **Execute**. This will add hydrogen atoms to the protein. This process is sometimes required even when the pdb file has hydrogens data.



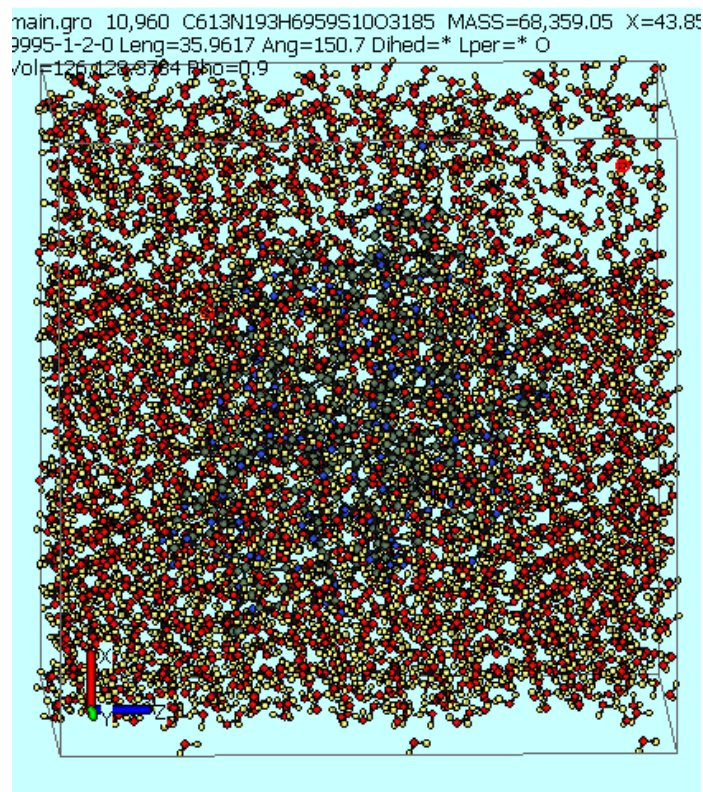
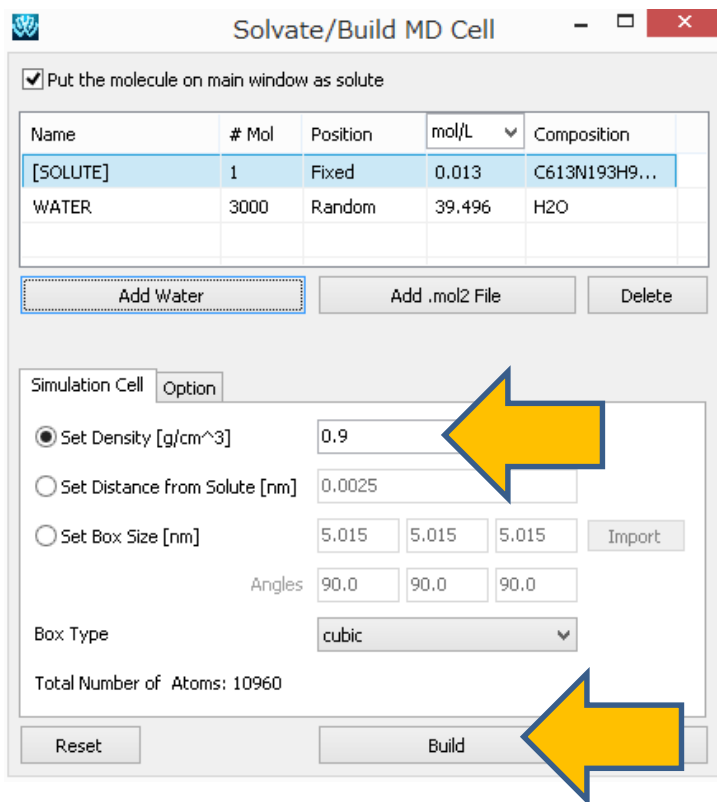
I. Build a simulation cell

1. Click **MD | Solvate/Build Cell**
2. Click **Add Water**, then set **Enter # of molecules** to **3000**, click **OK**.



I. Build a simulation cell

Set **Set Density** to **0.9**, click **Build** to build the simulation cell with water molecules.



I. Build a simulation cell

1. Click **MD | Generate Ions**.
2. On **Generate Ions** window, click **Execute**. Ions will be arranged and the system will neutralize.

MD Solid Tools Tutorial Help

Remote Job Submission

Solvate/Build Cell

Insert Molecules

Generate Ions

generated 10,900 Na11C613N193H6899S1003155Cl19 MASS=68,745.0
9995-1-2-0 Leng=10.6997 Ang=105.6 Dihed=* Lper=* O
Vol=126,128.3784 Rho=0.9051

Generate Ions

Commands for Cygwin:

```
echo "SOL" | gmx genion -s %OUTPUTNAME%.tmp.tpr -o %OUTPUTNAME%
```

Hide Detail

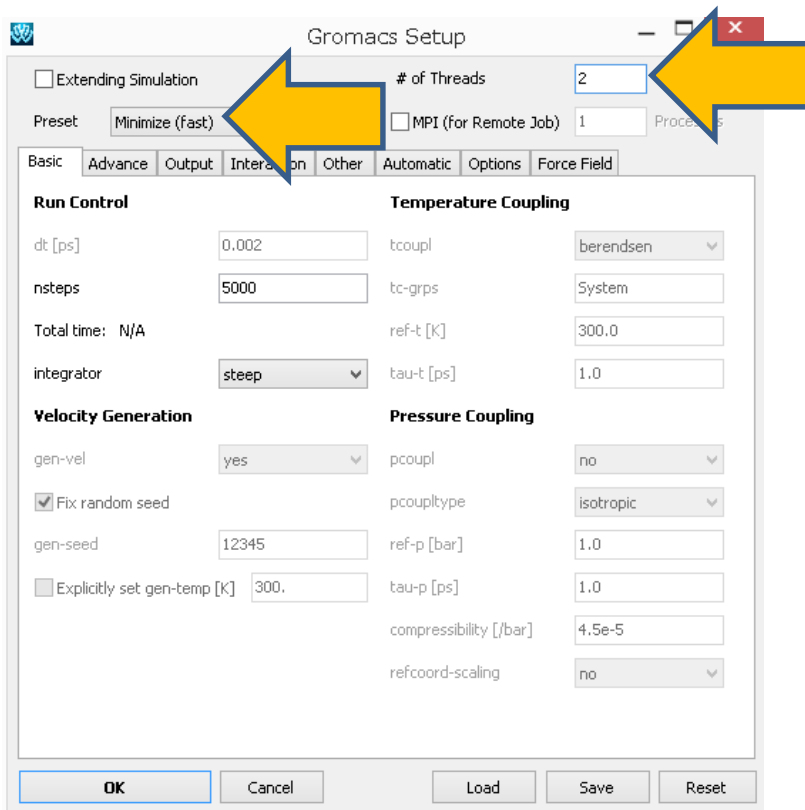
Execute

| Item | Value |
|-----------------------|-------|
| Neutral | True |
| Concentration [mol/L] | 0.15 |
| Cations | NA |
| Number of Cations | 0 |
| Anions | CL |
| Number of Anions | 0 |

II. Execute simulations

1. Equilibration (Energy minimization with restraint)

1. Click **MD | Gromacs | Keywords Setup**.
2. Set **Preset** to **Minimize (fast)**, **# of Threads** to a parallel number.
3. On **Advance** tab, check **-DPOSRES**, then click **OK**.



Gromacs Setup

Extending Simulation

Preset: **Minimize (fast)**

of Threads: **2**

MPI (for Remote Job) 1

Basic | **Advance** | Output | Interaction | Other | Automatic | Options | Force Field

Run Control

dt [ps]: 0.002

nsteps: 5000

Total time: N/A

integrator: steep

Velocity Generation

gen-vel: yes

Fix random seed

gen-seed: 12345

Explicitly set gen-temp [K]: 300.

Temperature Coupling

tcoupl: berendsen

tc-grps: System

ref-t [K]: 300.0

tau-t [ps]: 1.0

Pressure Coupling

pcoupl: no

pcoupltype: isotropic

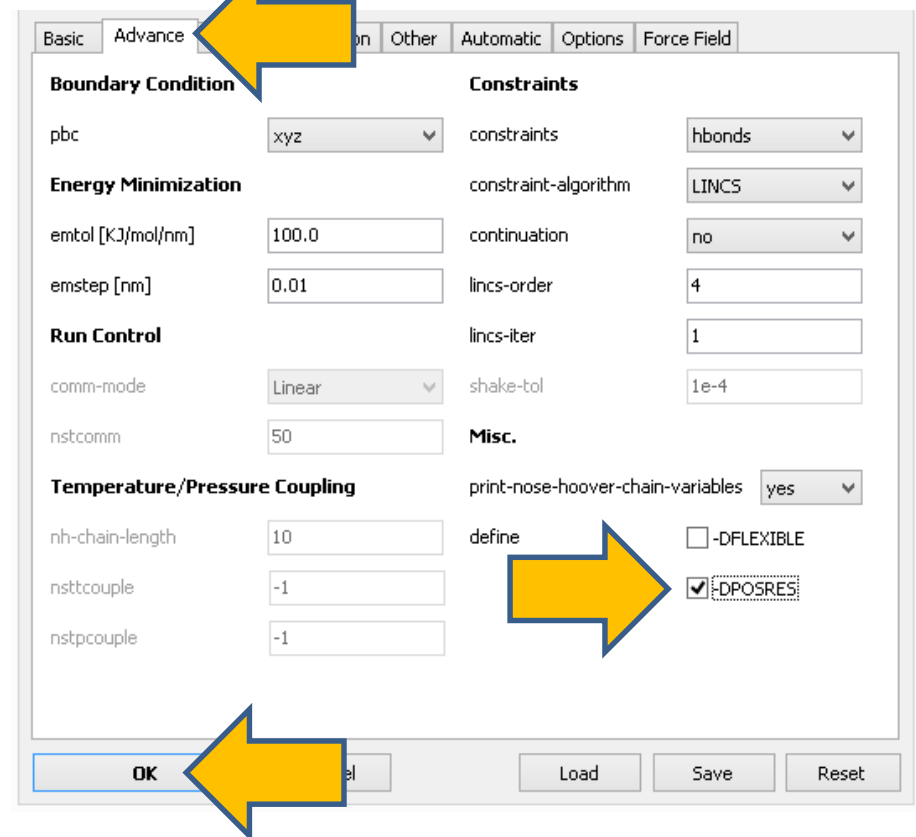
ref-p [bar]: 1.0

tau-p [ps]: 1.0

compressibility [1/bar]: 4.5e-5

refcoord-scaling: no

OK Cancel Load Save Reset



Basic | **Advance** | Output | Interaction | Other | Automatic | Options | Force Field

Boundary Condition

pbcs: xyz

Energy Minimization

emtol [KJ/mol/nm]: 100.0

emstep [nm]: 0.01

Run Control

comm-mode: Linear

nstcomm: 50

Temperature/Pressure Coupling

nh-chain-length: 10

nsttcouple: -1

nstpcouple: -1

Constraints

constraints: hbonds

constraint-algorithm: LINCS

continuation: no

lincs-order: 4

lincs-iter: 1

shake-tol: 1e-4

Misc.

print-nose-hoover-chain-variables: yes

define: -DFLEXIBLE **-DPOSRES**

OK Cancel Load Save Reset

II. Execute simulations

1. Equilibration (Energy minimization with restraint)

1. Click **MD | Gromacs | Start Gromacs**.
2. Save coordinate file as “**1AKI.gro**”, topology file as “**1AKI.top**”. This will launch **Winmostar JM** and start calculation on Cygwin.

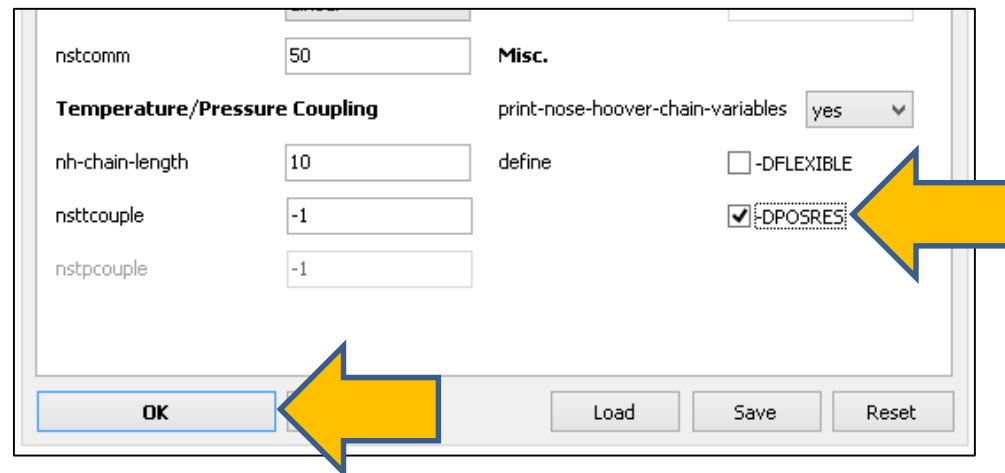
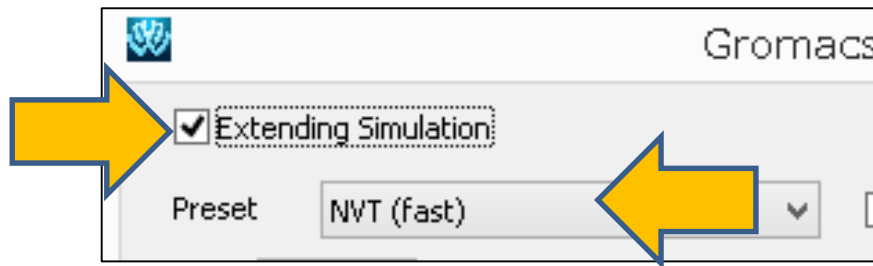
The image shows a software interface with a menu on the left and a terminal window on the right. The menu has the following items: MD, Solid, Tools, Tutorial, Help. Under MD, there are sub-items: Remote Job Submission, Solvate/Build Cell, Insert Molecules, Generate Ions, Assign Charges, Gromacs, and LAMMPS. The 'Gromacs' item is expanded, showing 'Keywords Setup' and 'Start GROMACS'. A blue arrow points from the 'Start GROMACS' option to the terminal window. The terminal window displays simulation output for steps 2333 to 2358, showing Dmax, Epot, and Fmax values.

| Step | Dmax | Epot | Fmax |
|------|------------|--------------|-----------|
| 2333 | 3.1e-03 nm | -1.81631e+05 | 1.34236e+ |
| 2335 | 1.8e-03 nm | -1.81633e+05 | 1.47046e+ |
| 2336 | 2.2e-03 nm | -1.81635e+05 | 1.91499e+ |
| 2337 | 2.7e-03 nm | -1.81637e+05 | 2.13751e+ |
| 2338 | 3.2e-03 nm | -1.81638e+05 | 2.73518e+ |
| 2339 | 3.8e-03 nm | -1.81639e+05 | 3.10191e+ |
| 2341 | 2.3e-03 nm | -1.81645e+05 | 4.05734e+ |
| 2342 | 2.8e-03 nm | -1.81649e+05 | 3.77722e+ |
| 2343 | 3.3e-03 nm | -1.81657e+05 | 1.27366e+ |
| 2345 | 2.0e-03 nm | -1.81659e+05 | 1.74990e+ |
| 2346 | 2.4e-03 nm | -1.81661e+05 | 1.88865e+ |
| 2347 | 2.9e-03 nm | -1.81662e+05 | 2.46743e+ |
| 2348 | 3.4e-03 nm | -1.81663e+05 | 2.76982e+ |
| 2349 | 4.1e-03 nm | -1.81663e+05 | 3.50483e+ |
| 2350 | 4.9e-03 nm | -1.81664e+05 | 4.03403e+ |
| 2352 | 3.0e-03 nm | -1.81673e+05 | 4.84224e+ |
| 2354 | 1.8e-03 nm | -1.81676e+05 | 2.23965e+ |
| 2355 | 2.1e-03 nm | -1.81680e+05 | 1.01263e+ |
| 2356 | 2.6e-03 nm | -1.81680e+05 | 2.90177e+ |
| 2357 | 3.1e-03 nm | -1.81685e+05 | 1.78256e+ |
| 2358 | 3.7e-03 nm | -1.81682e+05 | 3.84875e+ |

II. Execute simulations

1. Equilibration (*NVT* with restraint)

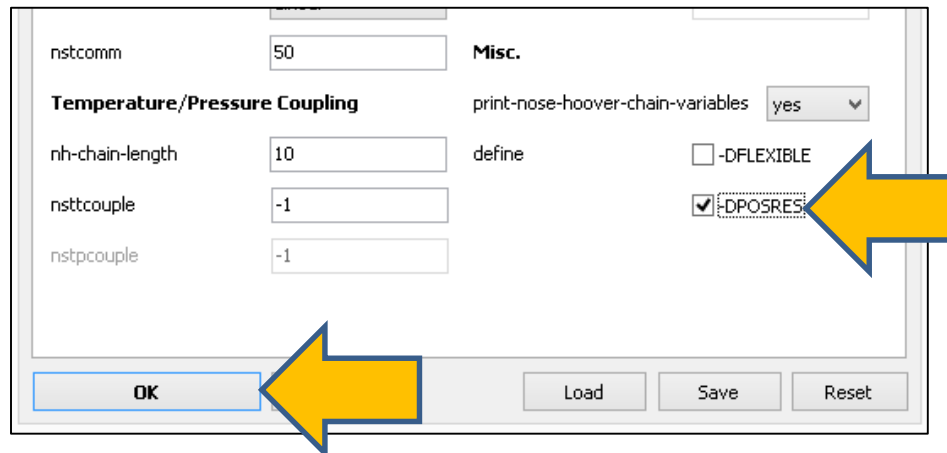
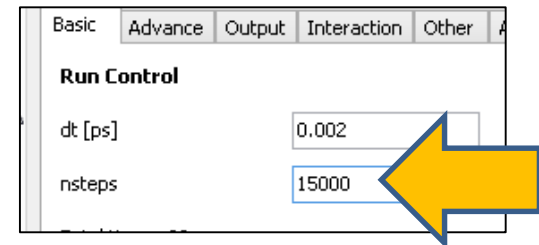
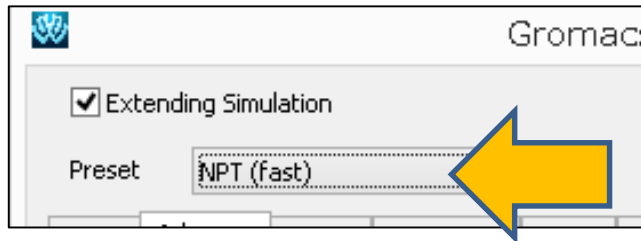
1. After the calculation, click **MD | Gromacs | Keywords Setup**.
2. Set **Preset** to **NVT (fast)**, check **Extending Simulation**.
3. On **Advance** tab, check **-DPOSRES**, then click **OK**.
4. Click **MD | Gromacs | Start Gromacs**.



II. Execute simulations

1. Equilibration (*NPT* with restraint)

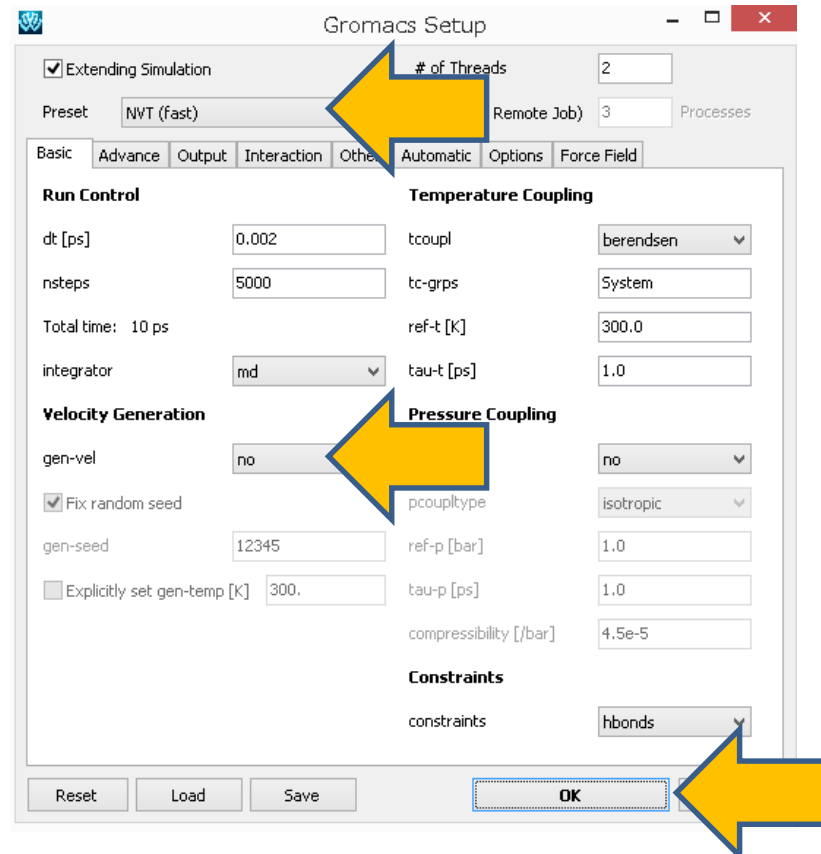
1. After the calculation, click **MD | Gromacs | Keywords Setup**.
2. Set **Preset** to **NPT (fast)**.
3. On **Basic** tab, set **nsteps** to **15000**.
4. On **Advance** tab, check **-DPOSRES**, then click **OK**.
5. Click **MD | Gromacs | Start Gromacs**.



II. Execute simulations

1. Equilibration (*NVT*)

1. After the calculation, click **MD | Gromacs | Keywords Setup**.
2. Set **Preset** to **NVT (fast)**, set **gen-vel** to **no**, then click **OK**.
3. Click **MD | Gromacs | Start Gromacs**. Calculation will start without any restraints.



II. Execute simulations

1. Equilibration (RMSD)

We've executed the calculation without restraints to the protein. So let us check the time changes of RMSD. This process should be carried out as necessary.

1. After the calculation, click **MD | Gromacs | RMSD**.
2. Open the default files. Repeat three times.
3. Set **Target Group** to **Backbone**, then click **Draw** to get the time changes of RMSD.

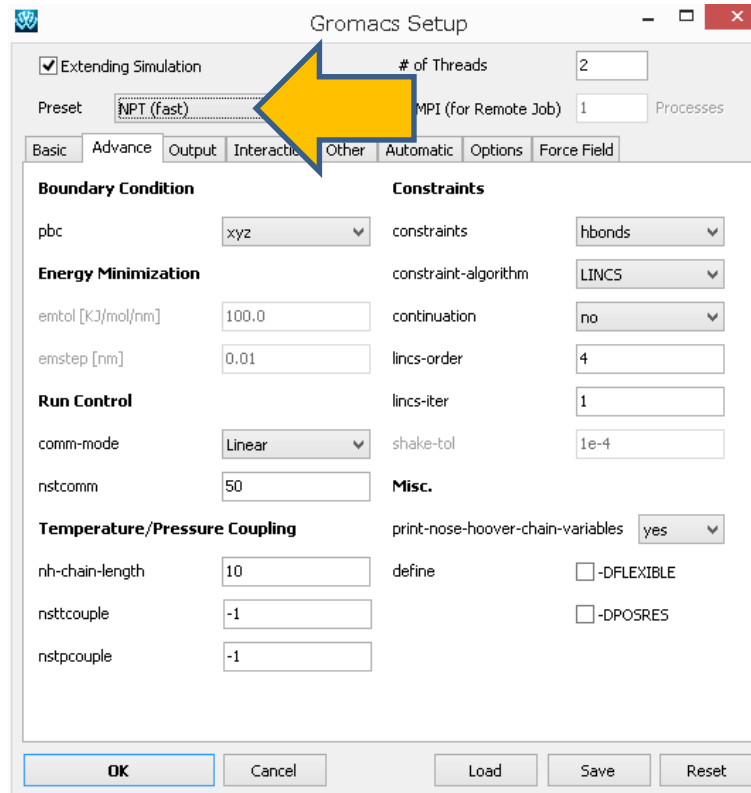
The radius of gyration can be obtained in the same way.

The screenshot displays the 'Root Mean Square Deviation' window in the software. The 'Target Group' dropdown menu is set to '4 : Backbone'. The 'First Frame [ps]' is set to '1.0'. The 'Draw' button is highlighted with a yellow arrow. The plot shows 'RMSD (nm)' on the y-axis (ranging from 0.04 to 0.065) and 'Time (ps)' on the x-axis (ranging from 0 to 10). The plot title is 'RMSD Backbone after lsq fit to Backbone'. A blue arrow points from the 'Gromacs' menu item to the 'Root Mean Square Deviation' option in the sub-menu. Another blue arrow points from the 'Draw' button to the plot area.

II. Execute simulations

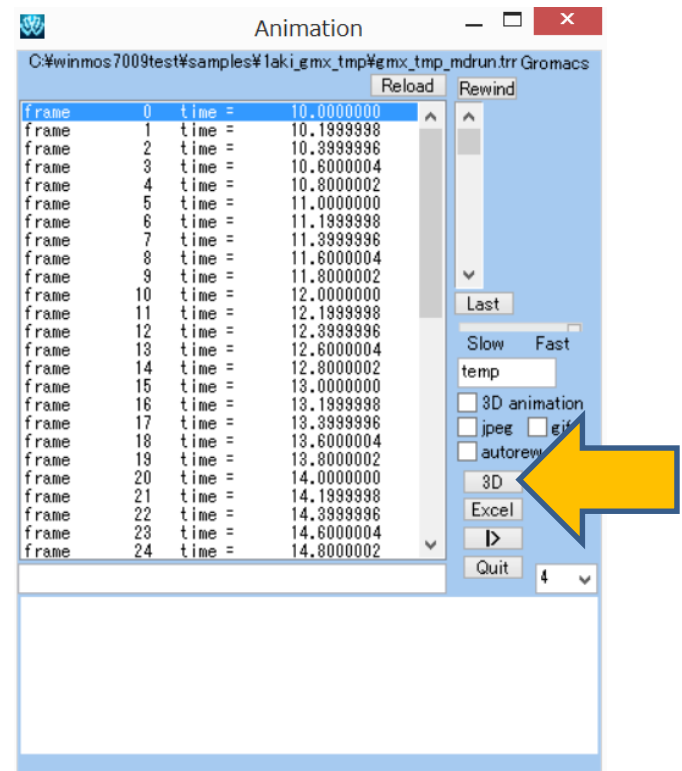
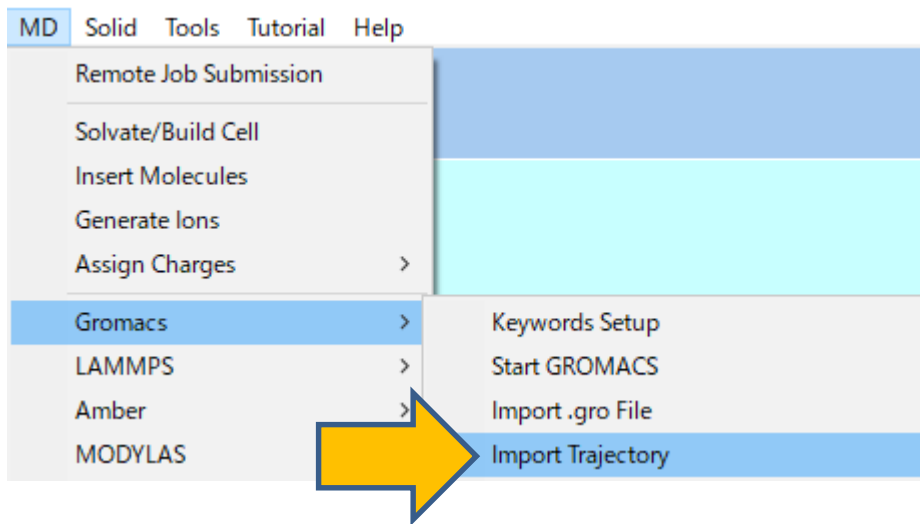
1. Equilibration (*NPT*)

1. After the calculation, click **MD | Gromacs | Keywords Setup**.
2. Set **Preset** to **NPT (fast)**, then click **OK**.
3. Click **MD | Gromacs | Start Gromacs**.



III. Product Run & Animation

1. After the calculation, click **MD | Gromacs | Start Gromacs**.
(The same condition as the last calculation for equilibration.)
2. After the product run, click **MD | Gromacs | Import Trajectory**.
Open the default files.
3. On **Animation** window, click **3D**.



III. Product Run & Animation

1. On **Winmostar 3D**, click **View | Preferences**.
2. Check **Mol. Weight** to change the view preferences to each atom.
3. Click **|>** (play button) in the upper-left box to start the animation.

The image shows the Winmostar 3D interface. On the left, the 'View' menu is open, with 'Preferences' highlighted. A yellow arrow points from this menu item to the 'Preferences' dialog box on the right. The dialog box has 'Rotation' set to 'Free', 'Boundary Condition' set to 'Mol.', and 'By turn' selected under 'Mol. Weight'. A red dashed box highlights the 'By turn' section, with a yellow arrow pointing to it. The main 3D view shows a complex molecular structure with atoms colored by weight. A blue arrow points from the 'View' menu area to the 3D view. In the top-left corner of the 3D window, there is a control box with a play button (|>) and other options like 'once', 'rew.', 'round', 'K', and 'I'. A yellow arrow points to this play button.