EvoS MD v1.0.0 (beta) | Windows 64-bit Version

File download: EvoS_MD_Setup_v1.0.0.exe

File size: 200 MB

Digitally unsigned

SHA-256: 959ED70AC2637A3B6010CF2C49046E135AE6C6ABAFFC43ED0F274E7AD61E25A4

Windows Installation Notice

This release does not include a digital signature. Microsoft Defender SmartScreen may therefore display an "unrecognized app" warning. To verify that the installer is the official software released by The Sound of Evolution, download EvoS MD only from the official The Sound of Evolution webpage and compare the SHA-256 value before installation. This software has undergone security scanning and was confirmed not to contain malware; users may proceed with installation.

Security Scan Record at Release

The installer was submitted to VirusTotal for analysis on May 27, 2026. Among 69 security detection engines, 68 detected no threat; Trapmine flagged the file as Suspicious.low.ml.score using a generic machine-learning rule. This result did not identify a specific malware family. The Sound of Evolution has retained the scan report and will continue to review and address possible false positives.

VirusTotal installer security scan: VirusTotal File Report

VirusTotal download-link security scan: VirusTotal URL Report

Introduction to EvoS Molecular Dynamics

EvoS MD is a graphical user interface (GUI) developed by The Sound of Evolution for operating GROMACS protein molecular dynamics simulations. GROMACS is an open-source molecular dynamics simulation program that is primarily operated through a command-line interface, with Linux remaining its mainstream working environment. EvoS MD is designed to help researchers establish, run, and analyse GROMACS molecular dynamics workflows in Windows through a more intuitive graphical interface. Users can import protein or protein-complex structure files with mouse-based operations, define the force field, water model, ionic environment, energy minimisation, equilibration and production dynamics conditions required for the simulation, and call GROMACS to perform the calculations.

EvoS MD is intended to help users manage and execute GROMACS workflows while lowering the barrier imposed by complex command-line operations, allowing molecular dynamics simulations to be applied more readily to studies of protein structure and interaction. In accordance with GROMACS guidance, simulation results must still be interpreted rigorously in light of study design, force-field selection, simulation duration and experimental evidence. A stable state observed in a single simulation cannot, by itself, serve as final proof of protein function or a genuine binding relationship.

Introduction to GROMACS

GROMACS is an open-source molecular simulation engine used for molecular dynamics (MD) simulations and energy minimisation. It represents proteins, nucleic acids, lipids, water molecules, ions and related systems through atom-scale models, and calculates interactions among atoms to follow the motion and structural changes of molecules over time. In biomolecular research, this approach can be used to observe protein conformational

fluctuations, stability at complex interaction interfaces, dynamic behaviour in solution and physical properties of molecular systems under different conditions.

Molecular dynamics is based on Newton's equations of motion. For a system composed of N atoms, the motion of atom i may be written as:

$$\mathbf{m_i \, (\partial^2 r_i / \partial t^2) = F_i, \quad i = 1, ..., N}$$

Here, m_i is the mass of atom i, r_i is its position, and F_i is the force acting on it. The force experienced by an atom is determined by the change in the potential function V with respect to position:

$$\mathbf{F_i = - \partial V / \partial r_i}$$

During a simulation, GROMACS repeatedly updates the position and velocity of each atom at very small time steps and records coordinates at regular intervals, creating a trajectory file that describes the dynamic history of the system. Once the system has completed its initial equilibration, investigators may use the trajectory to analyse molecular conformational changes, interatomic distances, hydrogen bonds, salt bridges, root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration and solvent-accessible surface area.

GROMACS calculations depend on a force field, which uses parameterised equations to approximate bonded interactions, electrostatic interactions and van der Waals forces between atoms. For large charged or hydrated biomolecular systems, long-range electrostatic interactions are commonly handled by methods such as Particle-Mesh Ewald (PME); simulation boxes generally employ periodic boundary conditions to avoid an unnatural external vacuum boundary around a finite water box. GROMACS also supports multicore parallel computation and high-performance computing architectures, enabling biomolecular systems containing large numbers of atoms to be simulated within practical time frames.

Molecular dynamics simulations nevertheless rely on approximate models. Atomic motion is primarily described through classical mechanics, while electronic excitation, electron transfer and chemical reactions generally fall outside the scope of ordinary classical molecular dynamics simulations. Force fields are also constrained by their parameters and modelling assumptions. Results produced by GROMACS should therefore be interpreted as computational analyses of molecular dynamics under defined models and conditions, together with experimental observations or other independent evidence.

System Requirements

The GROMACS version bundled as a backend for EvoS MD is GROMACS 2026.2 Prebuild (Windows, CUDA GPU Support, AVX2_256 AND AVX512). It therefore requires an NVIDIA GPU that supports CUDA 13.x and belongs to the Turing architecture or a later generation. GTX 10 series and older NVIDIA GPUs are outside the support range of this bundled GROMACS build.

EvoS MD can also be configured to use another version of GROMACS. See "Changing to a Different GROMACS Version" for operating instructions.

Hardware Item	Minimum Requirement
Operating system	Windows 10 or Windows 11, 64-bit
CPU	x86-64 processor with AVX2 support
System memory	16 GB or more; 32 GB or more recommended
GPU	NVIDIA CUDA GPU compatible with the bundled GROMACS CUDA build; GeForce RTX 2060 or equivalent is the minimum recommendation
GPU architecture	sm_75, sm_80, sm_86, sm_89, sm_90, sm_100, sm_120 or sm_121
VRAM	8 GB or more

Hardware Item	Minimum Requirement
Storage space	At least 2 GB for installation; an additional 10-50 GB or more is recommended for simulations
NVIDIA driver	NVIDIA driver supporting CUDA 13.x; minimum driver branch 580 or later; the latest stable version is recommended
Microsoft runtime	Microsoft Visual C++ v14 Redistributable x64

User Instructions

I. First-Time Functional Test of EvoS MD

Before beginning other work, follow the steps below to test whether EvoS MD operates correctly on your computer. If the procedure completes successfully, EvoS MD can execute on your system. If it cannot be completed, your computer does not meet the system requirements of EvoS MD.

Note: GROMACS is computationally demanding. While EvoS MD is operating, lag or a "not responding" state may occur during intensive computation. Please wait patiently and do not close the software directly, as temporary data may be lost.

Procedure

1. On the PDB + 3D View + Preflight page, click Load PDB files. Locate the original installation directory containing EvoS_MD.exe, open the Test folder and load the TEST.pdb file inside it.
2. On the 2. GROMACS Setup + Run page, click Browse under GROMACS executable and select Gromacs.2026.2.Prebuild.Windows.X64.CUDA13.0/bin/gmx.exe from the EvoS MD folder before running GROMACS. The Detect and Install GROMACS buttons can be ignored, as long as the executable path has been correctly specified. The project directory is the location where project files are stored and can be configured by the user.
3. The parameter settings may initially be left unchanged during this test.
4. Click Generate GROMACS project, followed by Run GROMACS. The execution time depends on the hardware. With an NVIDIA RTX 3060 graphics card, the green DONE state may be displayed after approximately half an hour, indicating that execution is complete. If FAIL appears during the process, the computer may not meet the system requirements of EvoS MD.
5. After GROMACS has finished running, click Play at the bottom of the PDB + 3D View + Preflight window to read the dynamic simulation result. On the Results + Analysis page, click Run GROMACS analysis to display analytical results obtained from the dynamic simulation.

Once the fifth step can be completed, other protein molecular dynamics simulations may be performed.

II. Main Window and Tab Structure

English Name	Description
PDB + 3D View + Preflight	Load protein structures, inspect the 3D model and perform input quality checks.
GROMACS Setup + Run	Specify GROMACS, configure simulation parameters and run simulations.
Results + Analysis	Generate, load and export analytical results; execute analysis commands.
Validation + Publication Report	Generate reports for individual simulation validation and software release validation.

English Name	Description
Research Workflow	Inspect or edit molecular dynamics parameters (MDP) content and execution scripts.
About + Licenses	View software information, third-party licences, contact information and the introduction webpage.

When the window is too small to display all contents in full, each major page provides scrolling functions. Users may reduce the window size and use horizontal or vertical scrollbars to inspect all fields and buttons.

1. PDB + 3D View + Preflight

This page is used to load protein structures, confirm the initial complex state, inspect input models and perform structural preflight checks before generating a GROMACS project.

English Name	Description
Language	Select English or Traditional Chinese from the drop-down menu to switch the interface display language. Most execution-process content is not available in Chinese.

1.2. PDB File Operations

English Name	Description
Load PDB files	Open a file-selection window and load protein structure files in .pdb or .ent format. Multiple files may be selected at once.
Remove selected	Remove the currently selected protein unit from the loaded list. The 3D viewer and preflight results are refreshed after removal. Select the protein to remove from Loaded PDB files below.
Loaded PDB files	Display the names, atom counts and residue counts of the currently loaded protein units.

Loading a PDB file containing multiple chains

When a single PDB file contains multiple protein chains, EvoS MD treats different chains as independent protein units and displays them in different colours in the 3D viewer. This is appropriate for processing a PDB file representing a completed docking result or a known complex structure.

1.3. Initial Pose Mode

This setting determines whether the loaded proteins retain their original relative coordinates before project generation or are rearranged automatically by the software.

English Name	Description
Auto separated (exploratory only)	Move the proteins apart to observe a separated state or test the viewer; this mode is unsuitable for formal complex-stability studies.
Near-contact (exploratory only)	Move the proteins into proximity for interface-operation testing or exploratory simulation; this does not represent a genuine binding pose.

English Name	Description
Keep original coordinates (research workflow)	Preserve the relative positions in the input PDB; this mode should be used for formal research applications.

1.4. Arrange and Preflight Check Buttons

English Name	Description
Auto Arrange Proteins	Rearrange the loaded proteins according to the currently selected initial-pose mode. If Keep original coordinates is selected, the original PDB positions are preserved.
Run Preflight Check	Check whether the loaded PDB contains problems that may block GROMACS project generation or a formal research workflow.

1.5. 3D Viewer

The 3D viewer is used to inspect the input proteins, the latest GROMACS structure or the complete trajectory. Different protein units are displayed in different colours.

Display check boxes

English Name	Description
Atoms	Display protein atom points or atom spheres.
Backbone	Display the protein backbone path.
Contact lines	Display auxiliary lines representing contact or proximity relationships between different proteins.

Contact lines are provided only for visual inspection. Interface stability should be evaluated together with analytical results such as distance, contact count and native contact fraction.

1.6. Style

English Name	Description
Backbone trace	Observe protein shape and relative position through backbone lines.
Ball-and-stick	Inspect local atomic arrangement using atom spheres and connecting bonds.
Approximate envelope	Inspect the overall protein outline and relative position through a simplified representation.
Atoms	Inspect the structure primarily through atom spheres.

1.7. Trajectory View

English Name	Description
Continuous interaction view (no-jump; dissociation inspection)	Observe whether proteins remain close, gradually move apart or display clear separation.

English Name	Description
Reference-anchored view	Fix the reference protein and observe the positional change of the other protein relative to it.
Aligned conformation view	Observe conformational change after alignment; this mode is unsuitable as a direct measurement of protein separation distance.
Bound-complex cluster view (inspection only)	Inspect cluster patterns; this mode is provided only for visual inspection.

1.8. Viewer Control Buttons

English Name	Description
Show input	Return to the originally loaded PDB structure view.
Latest GROMACS structure	Load the latest available GROMACS structure file from the current project.
Load trajectory	Read the protein trajectory from the current project and display it according to the Trajectory view selection.
Play	Play the currently loaded trajectory. After playback begins, the button changes to a pause function.
Pause	Pause the trajectory currently being played.
Reset view	Reset the viewer angle and zoom to their default settings.

Frame slider

Once a trajectory is loaded, drag the frame slider manually to the selected frame to inspect the protein position and conformation at that time point.

1.9. Preflight Results Table

After completion of the preflight check, the window displays a result summary and a problem table.

English Name	Description
Severity	Indicates whether the item is an Error, Warning or Info message.
Source	Indicates which input protein or project setting generated the issue.
Category	Indicates the type of issue, such as backbone continuity, initial pose or reproducibility.
Message	Describes the detected condition.
Suggestion	Provides the recommended direction for user action.

1.10. Meaning of Preflight Colours

Colour	Severity	Meaning
Red	Error	Blocks research-grade project generation and execution; the problem must be resolved first.

Colour	Severity	Meaning
Yellow	Warning	Operation may continue, but the user should read and document its effect.
Blue	Info	Loaded-result information or general information.

2. GROMACS Setup + Run

This page is used to specify the GROMACS executable, select the output folder, configure basic simulation parameters and start the simulation. When the number of fields exceeds the current window height, this page can be scrolled downward.

2.1. GROMACS Executable Settings

English Name	Description
GROMACS executable	Specify the Windows GROMACS executable to be called by EvoS MD, such as gmx.exe, gmx_mpi.exe or gmx_d.exe. To run the bundled GROMACS installation, load Gromacs.2026.2.Prebuild.Windows.X64.CUDA13.0/bin/gmx.exe inside the EvoS MD folder.

Action buttons

English Name	Description
Browse	Manually select a GROMACS executable on the computer.
Detect	Allow EvoS MD to attempt to detect GROMACS at the selected path, within the software installation location or in the system environment.
Install GROMACS	If the distribution package includes an installable Windows GROMACS archive, click this button to extract it and automatically detect the executable.

Compatibility note

EvoS MD v1.0.0 can connect to different Windows GROMACS executables. Whether a particular GROMACS build supports a specified GPU backend, force-field data or analysis command depends on the contents of that GROMACS build itself. For GROMACS operation and compatibility information, refer to the official documentation for the corresponding GROMACS version.

2.2. Project Directory

When a user executes a new simulation, EvoS MD creates a new independent run folder within the workspace, preventing the results of the previous task from being overwritten. Example:

```
C:/EvoS_MD_Project/
├─ run_20260526_201500/
├─ run_20260526_213040/
└─ run_20260527_093500/
```

2.3. Force Field and Water Model

English Name	Available Choices	Description
Force field	amber99sb-ildn, oplsaa, charmm27, gromos54a7	Select the force field used by GROMACS pdb2gmx.
Water model	tip3p, spce, tip4p	Select the solvent water model.

The choice of force field and water model must be appropriate for the research question, the protein system and the force-field data actually available in the GROMACS engine being used. For detailed principles of selection and parameter meanings, refer to the official GROMACS documentation and the relevant force-field literature.

2.4. Physical Environment Parameters

English Name	Default Value	Adjustable Range	Description
Temperature	310.15 K	250.0-400.0 K	Set the temperature parameter used for the simulation.
Pressure	1.0 bar	0.1-10.0 bar	Set the pressure parameter used for the simulation.
NaCl concentration (Added neutral NaCl pairs)	0.030 M	0.00-1.00 M	Set the concentration of NaCl added to the system.
KCl concentration (Added neutral KCl pairs)	0.120 M	0.00-1.00 M	Set the concentration of KCl added to the system.
Box distance	1.00 nm	0.60-4.00 nm	Set the distance between the protein and the boundary of the simulation box.

Salt-field description

EvoS MD provides separate NaCl and KCl concentration settings. Users may adjust the two values according to their experimental conditions. The software does not automatically determine whether a particular concentration combination is optimal for a specific cellular environment or protein system.

2.5. CPU and GPU Settings

English Name	Description
CPU threads	Set the number of CPU threads that GROMACS may use. If set to 0, thread allocation is determined by GROMACS.
Try GPU acceleration (verified at run time)	When the selected GROMACS build reports a GPU backend and candidate GPU hardware is present on the computer, EvoS MD is permitted to attempt GPU mode.

Automatic GPU determination

After the user specifies a GROMACS executable, EvoS MD reads version information from that executable and determines whether it reports a GPU backend such as CUDA, HIP, SYCL or OpenCL. If candidate hardware is detected, the GPU option may be selected automatically.

When the simulation is actually run, EvoS MD still performs a GPU runtime probe:

- If the GPU probe succeeds, the subsequent simulation may use the GPU.

- If the GPU probe fails, the program continues execution using the CPU.
- The actual execution mode adopted is recorded in GPU_RUNTIME_MODE.txt within the run folder.

2.6. Simulation-Stage Settings

English Name	Default Value	Adjustable Range	Description
Minimization steps	5000	100-1,000,000	Set the number of energy minimisation steps.
NVT	100 ps	0-100,000 ps	Set the duration of NVT equilibration.
NPT	100 ps	0-100,000 ps	Set the duration of NPT equilibration.
Production MD	1000 ps	0-1,000,000 ps	Set the duration of the production MD simulation. A basic research-grade setting is 100 ns (100000 ps) or longer.
Approximate number of frames per simulation	1000	1000 or 5000	Determine the approximate number of frames exported for the production trajectory.
Random seed	20260525	-1 to 2,147,483,647	Set the random seed used for velocity generation.

Guidance for the random seed

- Non-negative integer: recommended for formal research workflows because the value can be recorded and the initial velocity setting reproduced.
- -1: causes a random seed to be generated by the execution workflow and is recommended only for exploratory testing.

2.7. Structure Integrity Mode

English Name	Description
Research-grade intact protein	The default formal research mode. If a severe internal backbone discontinuity is detected, project generation may be blocked.
Exploratory TER gap auto-split	Allow an internal gap to be processed by TER-based segmentation. This mode is intended only for exploratory operation and should not be treated directly as evidence for binding stability of an intact protein.

2.8. Task and Execution Buttons

English Name	Description
Generate GROMACS project	Create an independent project folder, PDB, MDP and execution scripts from the current input structure and parameters, without immediately starting the simulation.
Run GROMACS	Create a new independent run folder and start the GROMACS simulation.
Stop	Stop the GROMACS execution process currently started by EvoS MD.
New task / Clear	Clear the currently loaded proteins, viewer trajectory, plots, log and validation display in preparation for loading another PDB dataset.
Open project folder	Open the current project folder.

2.9. Execution Status and Progress Display

English Name	Description
READY	No simulation is currently running.
Running...	GROMACS is performing the molecular simulation.
DONE	The GROMACS procedure has completed successfully.
FAIL	GROMACS execution has failed; an extractable reason for the failure is displayed below.

Display Element	Description
Progress bar	Updates the displayed percentage according to GROMACS workflow stages and readable log progress.
Failure-reason panel	If execution fails, displays an error summary extracted from the log.
Execution log panel	Displays standard-output and error messages generated during GROMACS execution.

The progress bar is an estimate generated by the GUI from workflow messages. Actual completion of the simulation must be determined from the GROMACS process termination state and generated output files.

3. Results + Analysis

This page is used to execute the standard analysis workflow, display analytical curves, export figures and data, and run additional post-processing commands through the restricted GROMACS Analysis Console.

3.1. GROMACS Results

A. Structural Stability

English Name	Output File	Description
Complex RMSD after fit (not separation)	rmsd.xvg	Display conformational deviation of the overall complex after alignment.
Complex RMSF	rmsf.xvg	Display fluctuations at positions throughout the complex.
Complex radius of gyration (Rg)	gyrate.xvg	Display changes over time related to the overall compactness of the complex.
Protein SASA	protein_sasa.xvg	Display solvent-accessible surface area of the protein over time.
Protein 1 C-alpha RMSD after self-fit (trajectory-derived)	protein1_ca_rmsd.xvg	Display conformational deviation of Protein 1 itself.
Protein 2 C-alpha RMSD after self-fit (trajectory-derived)	protein2_ca_rmsd.xvg	Display conformational deviation of Protein 2 itself.
Protein 1 C-alpha RMSF after self-fit (trajectory-derived)	protein1_ca_rmsf.xvg	Display fluctuations at C-alpha positions in Protein 1.
Protein 2 C-alpha RMSF after self-fit (trajectory-derived)	protein2_ca_rmsf.xvg	Display fluctuations at C-alpha positions in Protein 2.

B. Interface Stability

English Name	Output File	Description
Partner displacement RMSD after fit to protein 1	ppi_partner_relative_ca_rmsd.xvg	After Protein 1 is fixed by alignment, observe the change in relative position and orientation of Protein 2.
Protein-protein minimum distance (trajectory-derived)	ppi_mindist.xvg	Display changes in the distance between the closest atoms of the two proteins.
Protein-protein center distance (trajectory-derived)	ppi_com_distance.xvg	Display changes in the centre-to-centre distance between the two proteins.
Heavy-atom interface contacts <= 0.45 nm (trajectory-derived)	ppi_contacts.xvg	Display changes in the number of heavy-atom contacts satisfying the distance criterion.
Native heavy-atom contact fraction (trajectory-derived)	ppi_native_heavy_contact_fraction.xvg	Display the fraction of initial interface contacts retained in subsequent trajectory frames.

C. Simulation Quality Control

English Name	Output File	Description
Temperature vs time	temperature.xvg	Display the time series of simulation temperature.
Pressure vs time	pressure.xvg	Display the time series of simulation pressure.
Density vs time	density.xvg	Display the time series of system density.
Potential energy vs time	potential_energy.xvg	Display the time series of potential energy.

3.2. Protein Selection for Contact Analysis

English Name	Description
Contact analysis protein 1	Select the protein to serve as the first analysis target.
Contact analysis protein 2	Select the protein to serve as the second analysis target.

When an input structure contains two or more proteins or peptide chains, the user must select the two objects to be analysed. Derived results such as interface distance, contact count and native contact fraction are generated for this selected pair.

3.3. Standard Analysis Buttons

English Name	Description
Generate analysis script	Generate run_analysis.bat in the current run folder without executing it immediately.
Run GROMACS analysis	Generate and execute the standard analysis workflow; after completion, load the trajectory and the currently selected result.

English Name	Description
Load selected result	Load the currently selected .xvg file from the result drop-down menu and display it in the plot area.
Export plot PNG	Export the currently displayed analytical curve as a .png image.
Export data CSV	Export the currently displayed analytical data as a .csv file.
Export VMD trajectory PDB	Export the generated multi-model PDB trajectory as a .pdb file for reading by VMD or similar software.

Export VMD trajectory PDB requires md_vmd_pbc_protein_trajectory.pdb to exist in the current project folder.

3.4. Plot and Analysis Log Area

English Name	Description
Analysis plot	Display the currently selected .xvg result curve.
Analysis log	Display messages concerning analysis-script generation, GROMACS analysis execution, derived-metric calculation and result loading.

If the selected result file does not exist, the plot area clears the previous curve and Analysis log reports that the result is unavailable, preventing the user from incorrectly interpreting a previous result as the currently selected metric.

3.5. GROMACS Analysis Console

This function provides a restricted GROMACS analysis-command entry point, allowing users to execute additional GROMACS post-processing commands beyond the standard result menu. The console supports only analysis and trajectory post-processing functions; it does not accept arbitrary operating-system shell commands and is not intended to replace a complete GROMACS command-line workflow.

Console controls

English Name	Description
Command syntax / command field	Enter a GROMACS analysis subcommand, such as energy -f md.edr -o console_temperature.xvg.
Selection / stdin lines, one response per line (optional)	For GROMACS commands requiring interactive group selection, enter the selections to be supplied one per line.
Run console analysis	Execute the analysis command currently entered.
Stop console analysis	Terminate the analysis command currently running.
Console output	Display command-execution messages, error messages and information about newly generated result files.

Command input and Selection / stdin input

For example, the command field may contain the following subcommand:

```
energy -f md.edr -o console_temperature.xvg
```

Some GROMACS analysis commands require the user to select a group during execution. In this case, enter the response content in the stdin text box, with one response on each line. Multiple lines may be entered for multiple responses. For the energy command above, the stdin field may contain:

```
Temperature
```

3.6. Commands Allowed in the Console

The EvoS MD v1.0.0 console permits only the following GROMACS analysis or trajectory post-processing commands:

Command Group	Permitted Commands
Energy and overall analyses	energy, current, polystat, freevolume
RMSD / RMSF and conformation	rms, rmsf, rmsdist, gyrate
Distance and contact analyses	distance, pairdist, mindist, hbond, saltbr
Angles and geometry	angle, gangle
Principal components and clustering	covar, anaeig, cluster
Structure and secondary structure	dssp, mdmat, sasa
Radial and spatial analyses	rdf, spatial, vanhove
Trajectory processing	select, trajectory, trjconv, convert-trj, filter
Dynamic analyses	rotacf, velacc

For command parameters, group selection and the interpretation of output, refer to the official GROMACS Command-line Reference.

3.7. Console Safety Restrictions

The console does not accept the following shell operators:

```
|    &&    ||    ;    >    <    `
```

3.8. Five Console Test Examples

The following tests should be run only after the GROMACS simulation has completed and the current project folder contains md.tpr, md.xtc and md.edr.

Test Objective	Command Field	Selection / stdin Field	Expected Result
Temperature curve	energy -f md.edr -o console_temperature.xvg	Temperature	Generate a temperature time-series plot.
Potential-energy curve	energy -f md.edr -o console_potential.xvg	Potential	Generate a potential-energy time-series plot.
RMSD	rms -s md.tpr -f md.xtc -o console_rmsd.xvg	Line 1: Protein Line 2: Protein	Generate an RMSD curve.
Radius of gyration	gyrate -s md.tpr -f md.xtc -o console_gyrate.xvg	Protein	Generate a radius-of-gyration curve.

Test Objective	Command Field	Selection / stdin Field	Expected Result
Minimum distance	mindist -s md.tpr -f md.xtc -od console_mindist.xvg	Enter two protein groups according to the group names listed in Console output.	Generate a minimum-distance curve.

4. Validation + Publication Report

This page is used to organise validation information relating to simulation files, parameters, output results and the software execution environment.

4.1. Validation of an Individual Simulation

English Name	Description
Run validation checks	Check the parameters, required output files, simulation duration, structure mode, initial-state source and analytical-result completeness in the current run folder.
Export validation report	Export the current validation report as a text file.
Validation report	Display the current run validation contents and warnings.

Items inspected by the validation report

- The execution script used by the GROMACS project.
- Whether MDP files and important output files are present.
- Whether the expected Production MD duration agrees with the actual end time of the analysis.
- Whether the intact-protein research mode was used.
- Whether the original coordinates of the input complex were preserved.
- Whether an explicitly recorded random seed was used.
- Whether the trajectory quality check contains abnormal warnings.
- Whether all required analytical results have been generated.
- Whether the derived-metric implementation audit passed.

4.2. Software Release Validation Center

English Name	Description
Run release validation	Generate the release-validation contents and display them in the text box.
Export release validation package	Create a validation-package folder and export reports, the hash manifest and important evidence files.

Contents of the release-validation package

The exported validation package may include:

```
SOFTWARE_RELEASE_VALIDATION_REPORT.txt
RUN_VALIDATION_REPORT.txt
RUN_FILE_SHA256_MANIFEST.txt
combined_complex.pdb
```

```
topol.top
minim.mdp
nvt.mdp
npt.mdp
md.mdp
run_project.bat
run_analysis.bat
rmsd.xvg
rmsf.xvg
gyrate.xvg
protein_sasa.xvg
ppi_contacts.xvg
ppi_mindist.xvg
ppi_com_distance.xvg
INTERACTION_TRAJECTORY_QUALITY_CHECK.txt
DERIVED_METRIC_IMPLEMENTATION_AUDIT.txt
INITIAL_POSE_PROVENANCE.txt
STRUCTURE_INTEGRITY_MODE.txt
PRECHECK_REPORT.txt
GPU_RUNTIME_MODE.txt
```

Scope of interpretation of the validation report

The Validation Center is intended to confirm that:

- The software execution environment can be recorded.
- The current GROMACS executable can run.
- Required run files and analysis outputs are complete.
- The workflow checks built into the GUI have passed.

A validation report does not, by itself, demonstrate that two proteins genuinely interact in a living organism, nor does it alone guarantee that a research conclusion is sufficient for publication.

5. Research Workflow

This page provides more advanced project and MDP (molecular dynamics parameters) management functions. General users may complete the standard workflow using the default parameters on the GROMACS Setup + Run page; users familiar with GROMACS may inspect or modify MDP contents on this page.

Before customising an MDP file, confirm that you understand the physical meaning, compatibility and research consequences of each GROMACS parameter. For details, refer to the official GROMACS .mdp Options Documentation.

5.1. Controls

English Name	Description
Use custom MDP text	When selected, use the MDP content in the editors on this page when generating a project or exporting a command bundle.
Load current generated MDP templates	Regenerate and load the MDP templates for each stage according to the current settings on the GROMACS Setup + Run page.
Refresh command preview	Regenerate the text preview of the Windows, Linux and analysis scripts.
Export command bundle	Export MDP files and execution scripts to the selected folder.

5.2. MDP Editor Tabs

Tab Name	Description
minim.mdp	MDP content for the energy minimisation stage.
nvt.mdp	MDP content for the NVT equilibration stage.
npt.mdp	MDP content for the NPT equilibration stage.
md.mdp	MDP content for the production MD stage.
Command preview	Display the planned Windows execution script, Linux execution script and analysis script.

5.3. Export Command Bundle

After Export command bundle is clicked, EvoS MD exports the following files to the folder selected by the user:

```
run_project.bat
run_project.sh
run_analysis.bat
minim.mdp
nvt.mdp
npt.mdp
md.mdp
validation_report_template.txt
```

This function is suitable for:

- Preserving the actual MDP settings used in research.
- Providing a reference for another computer or for a command-line workflow.
- Serving as part of a record of research methods and reproducibility.

6. About + Licenses

This page displays EvoS MD development information, contact details, third-party component licences and the software-introduction website.

III. Changing to a Different GROMACS Version

The Windows GROMACS version bundled by default with EvoS MD is Gromacs.2026.2.Prebuild.Windows.X64.CUDA13.0. This folder is located in the same directory as the original EvoS_MD.exe file.

A replacement GROMACS version must likewise support Windows. Place the downloaded GROMACS version in the same directory, then select the gmx.exe file of the desired GROMACS version in the GROMACS executable field on the GROMACS Setup + Run page of EvoS MD. Subsequent operations can then be performed using that selected GROMACS executable.

IV. Typical Complete Operating Example

The following workflow applies to a two-protein complex with a known or previously established initial binding pose.

Step 1: Load the Protein Structure

- Open the 1. PDB + 3D View + Preflight page.
- Click Load PDB files.
- Select a PDB containing two protein chains.
- Confirm that different protein units are displayed in different colours in the viewer.

Step 2: Set the Initial State

- Select Keep original coordinates (research workflow) under Initial pose mode.
- When the input file already contains the correct complex position, do not use the exploratory auto-separated or near-contact arrangement modes.

Step 3: Perform the Preflight Check

- Click Run Preflight Check.
- Read the Error and Warning items in the table.
- If an Error exists, correct the structure or settings and perform the check again.

Step 4: Configure GROMACS

- Open the 2. GROMACS Setup + Run page.
- Use Browse to specify a usable Windows GROMACS executable, or use Detect.
- Specify the project-folder location.
- Set the force field, water model, temperature, pressure, NaCl, KCl, box distance, equilibration and production MD duration. Default values may be used as a reference.
- For research requiring reproducibility, specify a defined non-negative random seed.

Step 5: Run the Simulation

- Click Run GROMACS.
- Observe the status panel, progress bar and log.
- Wait until the status changes to DONE. Higher parameter values require longer execution times; for example, setting Production MD to 10000 ps may require approximately one day on an NVIDIA RTX 3060 graphics card.
- If the status changes to FAIL, read the failure reason and log before addressing the problem.

Step 6: Perform Analysis

- Open 3. Results + Analysis.
- Select Contact analysis protein 1 and Contact analysis protein 2.
- Click Run GROMACS analysis.
- Load the desired curve from the GROMACS result menu.

Step 7: Inspect the Trajectory

- Return to 1. PDB + 3D View + Preflight.
- Under Trajectory view, select Continuous interaction view (no-jump; dissociation inspection).
- Click Load trajectory.
- Use Play or the frame slider to observe changes in protein position.

Step 8: Export Results

The 3. Results + Analysis page can export:

- Plot PNG files.
- Data CSV files.
- VMD trajectory PDB files.

Step 9: Generate a Validation Report

- Open 4. Validation + Publication Report.
- Click Run validation checks.
- Read the validation contents and confirm that the required results are present.
- Click Export validation report.
- For formal release or method-acceptance materials, perform validation and package export through the Software Release Validation Center.

V. Recommendations for Result Preservation and Data Management

Each simulation creates an independent run folder. For formal research use, preservation of the following data is recommended:

Category	Files to Preserve
Input structure	Original PDB and combined_complex.pdb
GROMACS settings	topol.top, minim.mdp, nvt.mdp, npt.mdp and md.mdp
Execution scripts	run_project.bat and run_analysis.bat
Primary trajectory and system files	md.xtc, md.tpr and the required trajectory PDB files
Analysis results	.xvg files and exported .csv and .png files
Workflow evidence	PRECHECK_REPORT.txt, INITIAL_POSE_PROVENANCE.txt, STRUCTURE_INTEGRITY_MODE.txt and GPU_RUNTIME_MODE.txt
Validation data	Validation report and validation package

VI. Limitations of Use and Considerations for Research Interpretation

1. EvoS MD performs simulations according to the input structure and GROMACS settings; it does not independently demonstrate that the state of the input complex is a genuine binding state.

2. Complex RMSD after fit primarily reflects conformational change in the aligned complex and should not be used alone to determine whether two proteins have separated.

3. When observing protein-interface stability, the following should be examined together:

- Partner displacement RMSD after fit to protein 1
- Protein-protein minimum distance
- Protein-protein center distance
- Heavy-atom interface contacts
- Native heavy-atom contact fraction
- Trajectory visualisation results

4. Exploratory modes that automatically arrange proteins are suitable only for interface testing or preliminary exploration.

5. For research-oriented simulations, preserve the source of the initial state, force field, water model, MDP files, random seed, GROMACS executable version and the necessary replicate design.

6. For details regarding custom MDP files, additional console analysis commands, GPU-backend behaviour and GROMACS error messages, refer to the official GROMACS documentation and relevant methodological literature.

GROMACS Documentation Note

EvoS MD provides a graphical operating workflow. Detailed usage of GROMACS commands and simulation parameters is governed by GROMACS itself. For full command and parameter descriptions, consult the official GROMACS Command-line Reference and Molecular Dynamics Parameters (.mdp options).