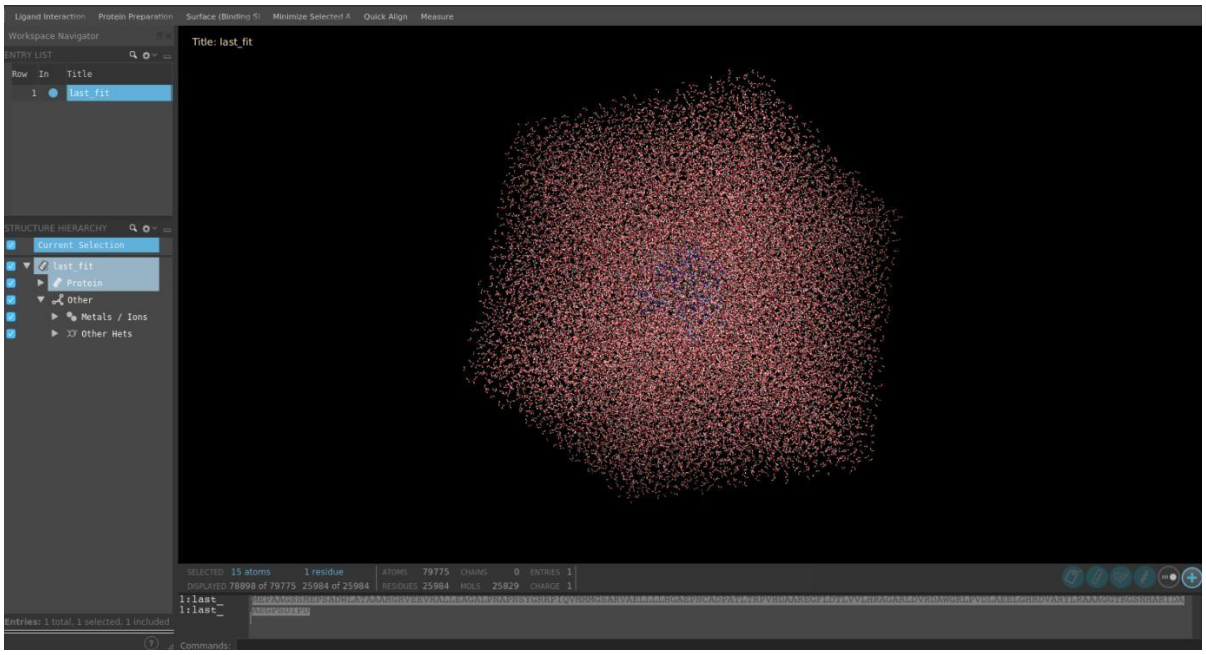
Mutation of the structure in Maestro (P48L/H83Y)

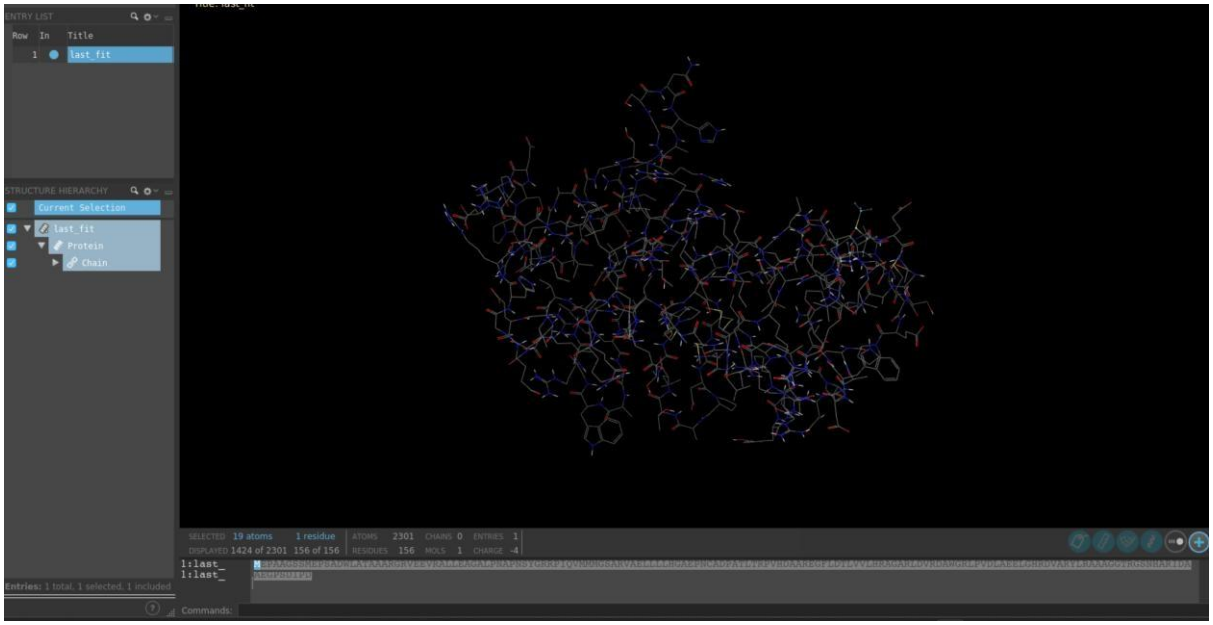
1. Opening the structure

Launch Maestro.
Load the last frame of the MD trajectory via **File → Import Structures** (for example, `last_fit.pdb`).
Make sure the structure appears in the **Workspace Navigator** (entry `last_fit`).



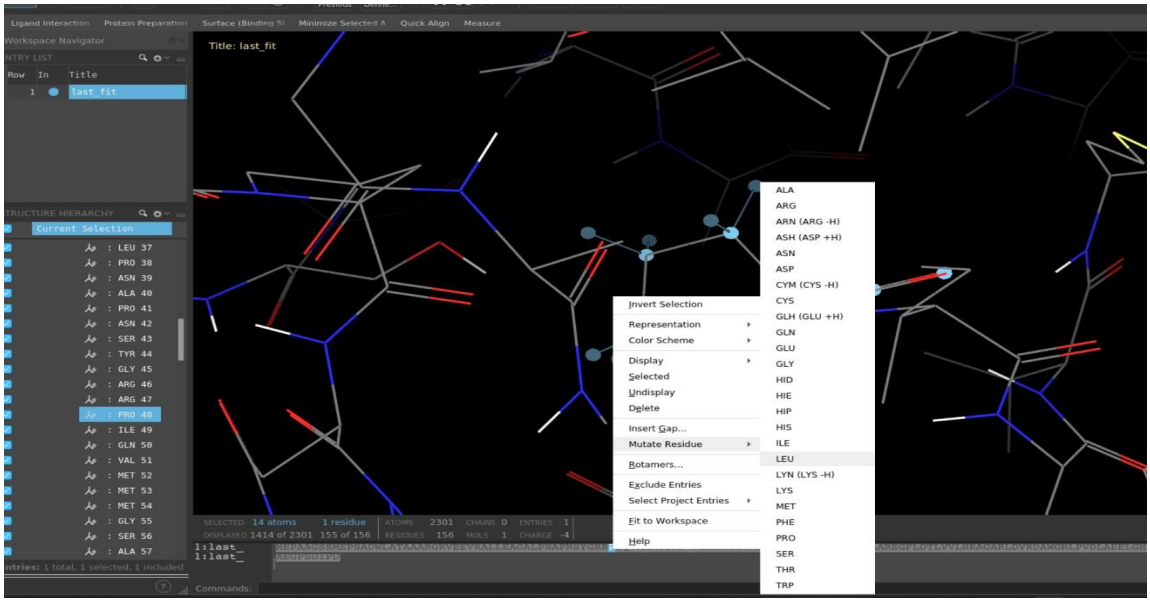
2. Removing unnecessary molecules (water, ions)

In the **Structure Hierarchy** panel, expand:
`last_fit → Other → Metals / Ions, Other Hets`
Select water molecules and ions, right-click, and choose **Delete Atoms**.
(Only the protein remains in the workspace.)



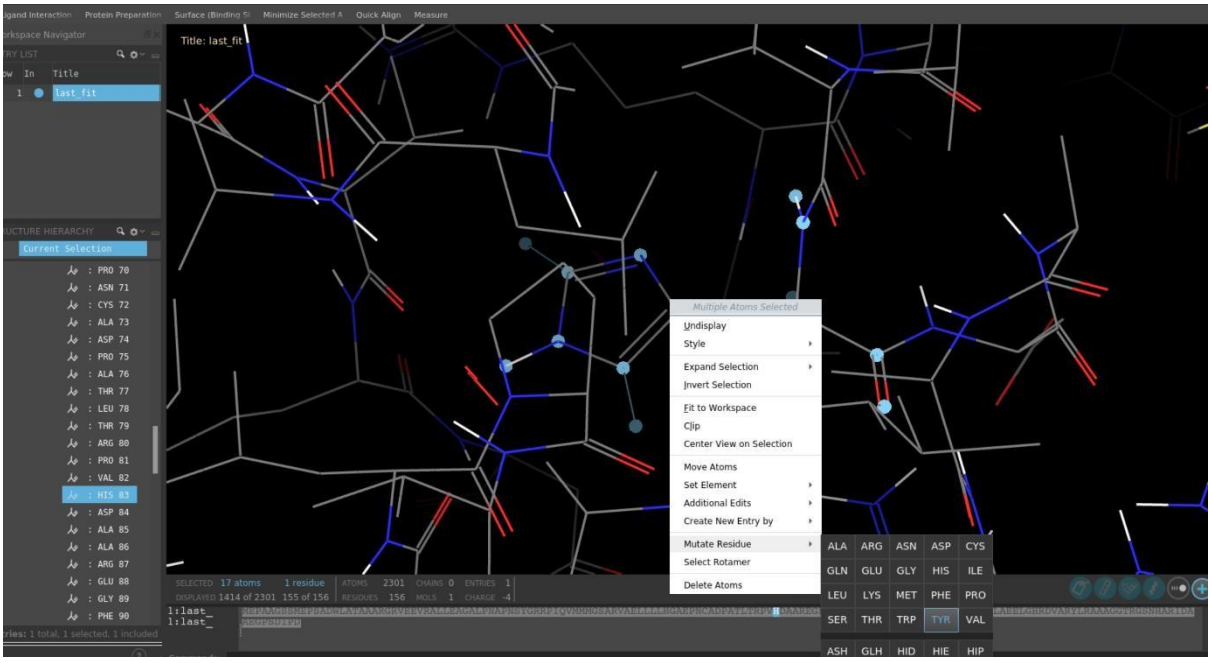
3. Mutation of PRO48 → LEU (Method 1 — via the residue list)

In the **Structure Hierarchy**, navigate to:
`last_fit → Protein → Chain`
Scroll through the list to **PRO 48** and click on it (the residue will be highlighted).
Go to the highlighted residue in the sequence (P), select **Mutate Residue**, choose **LEU** from the amino acid list, and confirm the mutation.
Maestro will automatically rebuild the side chain.



4. Mutation of HIS83 → TYR (Method 2 — via the 3D structure)

In the 3D window, right-click directly on an atom or the side chain of residue **HIS 83** to select it.
After selecting the residue, choose **Mutate Residue**, specify the amino acid **TYR**, and confirm the mutation.



5. Checking the mutations

Make sure that:

- Position 48 — LEU
- Position 83 — TYR

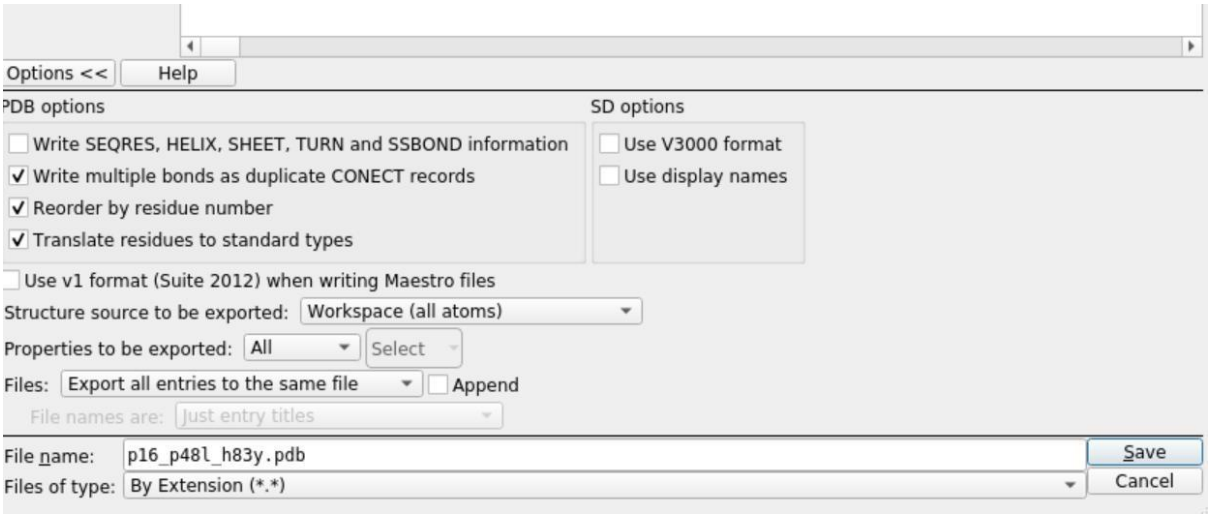
6. Local minimization of the mutated residues

Select only the mutated residues (**P48** and **H83**) and run **Minimize Selected Atoms** (on the top toolbar) to relieve local strain without altering the global protein structure.

After performing local minimization of the mutated residues, no additional manual structural adjustments are required. At the next stage, the system will be re-prepared and fully relaxed during minimization, heating, and the production molecular dynamics run in GROMACS, which will automatically remove residual steric strain and optimize the protein conformation in explicit solvent conditions.

7. Saving the structure

In the menu, go to **File → Export Structures** to open the export window.



In the export settings, leave the default values and make sure that **Workspace (all atoms)** is selected as the source.
At the bottom of the window, in the File name field, specify the **file name**, for example:
p16_p48l_h83y.pdb
From the format drop-down menu, select **PDB**, then click **Save**.