Tutorial Two: Relax Structure, Scatter Plot, Minimize Structure

Estimated time: 20 minutes

This tutorial will instruct users how to generate optimized (low energy) protein conformations using the Relax and Minimize Actions, and the Scatter Plot tool.

In order to run this tutorial, you will need to know how to use the tools from Tutorial One.

(Caveat: The estimated time does not include the time it takes for jobs to run on the server. There will be several pauses while you wait 20 minutes or more for structures to run on the cloud before you can move on to more steps.)

1) Introduction to Running Relax

Relax is an aggressive Rosetta tool for sampling multiple conformational changes in a protein to find a structure with the lowest Rosetta Energy. To read more about Relax click here. Relax is the best way to find the lowest energy conformation for your structure. Since all our tools will use energy for optimization, using the lowest energy structure for those tools will decrease the noise and improve their accuracy.

To find the structure with the lowest energy, multiple rounds of Relax will be run. The scores for the structures will decrease as more low energy structures are generated and the range of scores will narrow. After each round of Relax the best structure or structures will be selected to use as the starting structures for another round of Relax. When the scores of structures can no longer get lower, an optimized structure has been generated (which is the structure with the lowest energy).

To run Relax, first select a Project or Create a Project and load the structure 5KIZ. In Tutorial One we recommended running Prepare on a new structure after loading it into Bench. However Relax is a more aggressive version of Prepare so we can skip it here.

After the structure has loaded, click on the 5KIZ folder in the left window in order to bring it into the center tab.
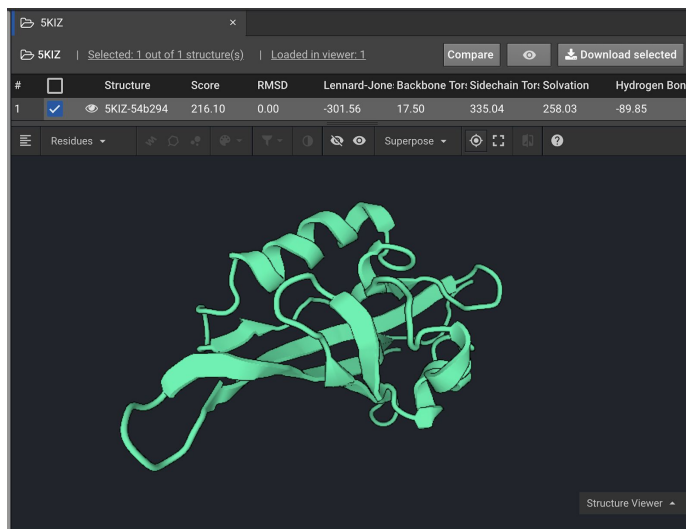
Click the ☑ to the left of the structure name in order to select it. Then, in the right window, click Relax . This will open a Relax tab in the center window. Enter Repeats ⓘ [5] and click ⚙ Save & Run .

For this tutorial, we only have you run 5 repeats to limit your credit usage, but we normally recommend 20 repeats in order to get enough diversity in your results to make significant improvement. This should take twenty minutes or more depending on how many jobs are in the Queue ahead of it.

Once the Relax has run, it will appear in the left window. Click the 🖉 to the right of the Relax results file and change the name to *relax 1 5KIZ*. Click on the Relax folder in order to open it's tab in the center window. Structures will be listed in the order that they were created and labelled structure 1 to structure 5. You can reorder the structures by any of the columns:

#	☐	Structure	Score	RMSD	Lennard-Jones	Backbone Torsion	Sidechain Torsion	Solvation	Hydrogen Bond

Click on Score . The structure with the lowest energy will be listed first. In the example run shown below the structure with the lowest energy had a score of -187. Click Score again to reverse the order of scores; the structure with the highest energy will now be listed first.

#	☐	Structure	Score	RMSD	Lennard-Jones	Backbone Torsion	Sidechain Torsion	Solvation	Hydrogen Bond
1	☐	structure 1	-181.69	1.32	-393.37	-5.19	114.98	244.12	-136.77
2	☐	structure 2	-187.43	1.08	-394.13	-6.02	112.88	243.31	-138.03
3	☐	structure 3	-183.18	1.18	-393.27	-4.87	110.75	243.59	-133.95
4	☐	structure 4	-187.09	1.16	-392.27	-4.89	109.53	242.34	-136.46
5	☐	structure 5	-178.40	1.35	-394.50	-4.51	116.87	243.58	-134.36

Rank by score to find lowest energy structure and select it as best structure

#	☐	Structure	Score ∧	RMSD	Lennard-Jones	Backbone Torsion	Sidechain Torsion	Solvation	Hydrogen Bond
1	☑	structure 2	-187.43	1.08	-394.13	-6.02	112.88	243.31	-138.03

2) Selecting One or Multiple Low Energy Structures with Scatter Plot

From the 5 structures created with Relax, click the ☑ to the left of the structure name with the lowest energy. Then, in the right window, click Relax . This will open a Relax tab in the center window. Enter Repeats ⓘ [5] and click ⚙ Save & Run . This will run another Relax using the best structure as input.

This is extra round of Relax is often needed to find the global minimum. Very small structures will not get an improved score. Structures at ~400 residues will likely find the minimum after this second round of Relax, but larger structures could need multiple rounds of Relax. You know that you have done enough Relax rounds when the score stops getting better.

Optional: Use multiple input structures for second round of Relax

For 99% of the time, you will want the lowest energy structure from a Relax to use as the input for another round of Relax. However, it is possible that you want to allow more structure diversity as input for more Relax. This is useful when you are not sure whether your structure is close to optimal. In this case, select structure with larger rmsd in order to broaden the search space.

While you are still in the Relax tab for your finished Relax, click [Structure Viewer ▲] in the lower center window. Change the button to [Scatter Plot ▲]. By default, Scatter Plot displays the Score on the Y axis and the RMSD (compared to the starting structure) on the X axis:
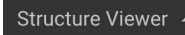
Y Axis: Score ▼	X Axis: RMSD ▼

However, you can change either of these axes to Score, RMSD, or to individual energy terms. From the default Scatter Plot setting, select a couple structures (3 or 4 structures) that have the lowest energy, but have a broad range of RMSD. You can do this by click & dragging a box around the dots that are lowest in the plot:
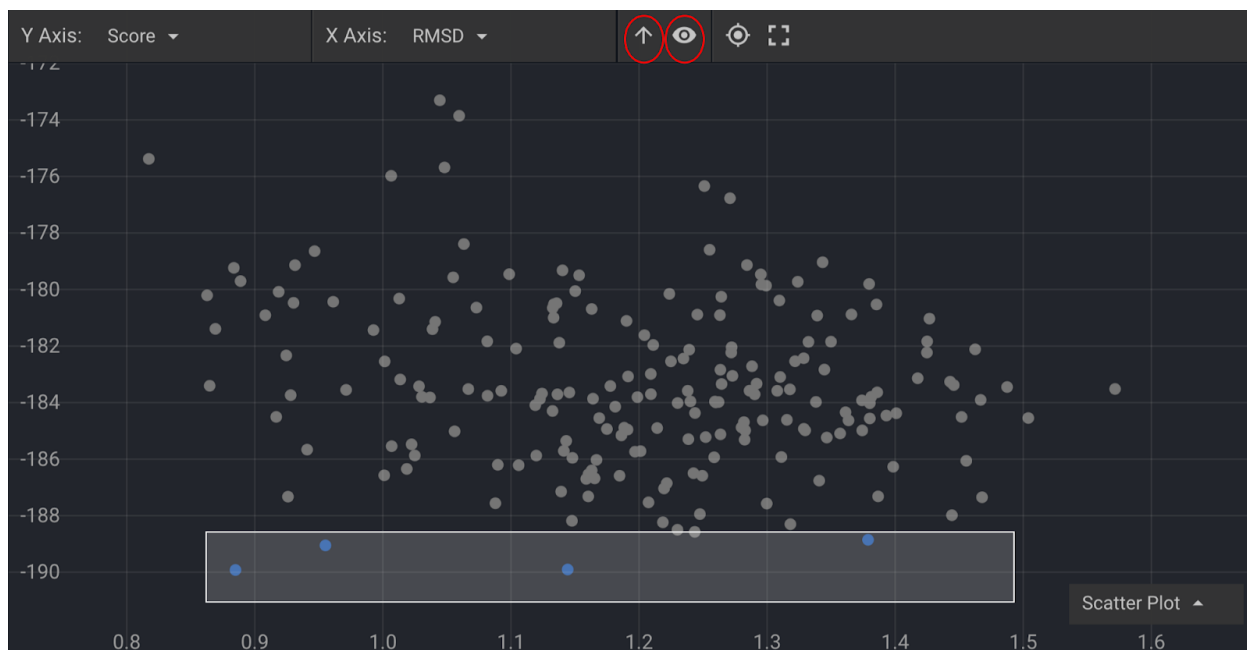
(Note: The above example shows the results from 200 repeats of Relax which is normally more than is necessary)

These selections will turn blue. You can work with these selections by clicking either of the icons highlighted with red circles in the image above. Click ⬆ for the selected structures in the plot to become checkmarked in the list above.

These structures will be used for the next round of Relax because they are energetically favorable and structurally diverse. This is best done from the Scatter Plot view so that you can be certain that you are selecting a diverse sampling.

Before starting the next round of Relax, click on the 👁 in order to visualize the areas of structural diversity. This activates the 👁 above for these structures. Click `Scatter Plot ▲` and change this button to `Structure Viewer ▲`. Select the entire structures for your three or four structures in the Structure Viewer (see Tutorial One if needed). Click `Superpose ▾` and select `Selected backbone atoms`:
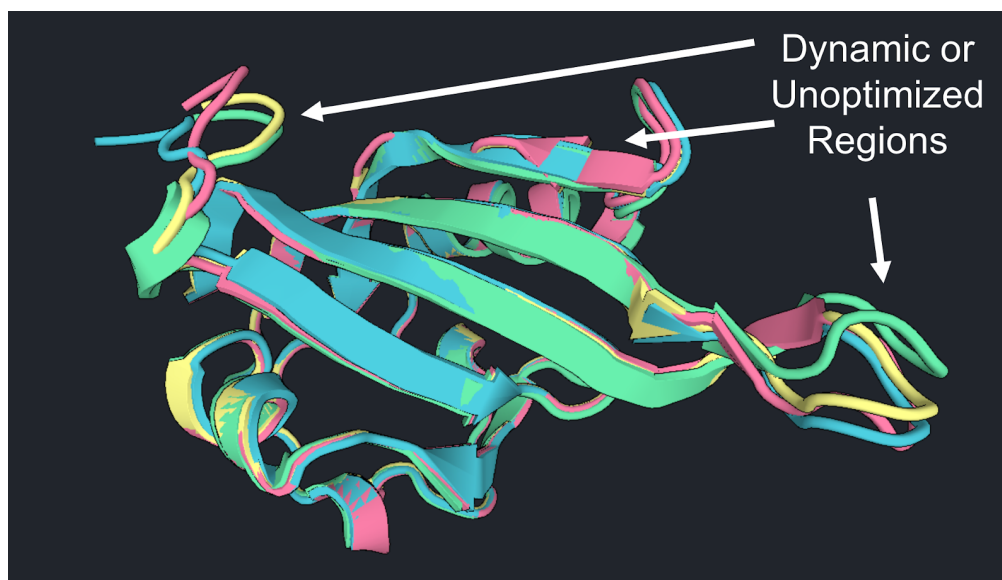
You can see above that the major differences in these structures are in two loop regions and a region of beta sheets.

The three or four structures checkmarked from the previous step will be used as starting structures for Relax iterations in order to find the structure with the energetic minimum.

Click [Relax] to open a Relax tab in the center window. You will note that the four example structures selected from the Scatter Plot are listed below as starting structures.
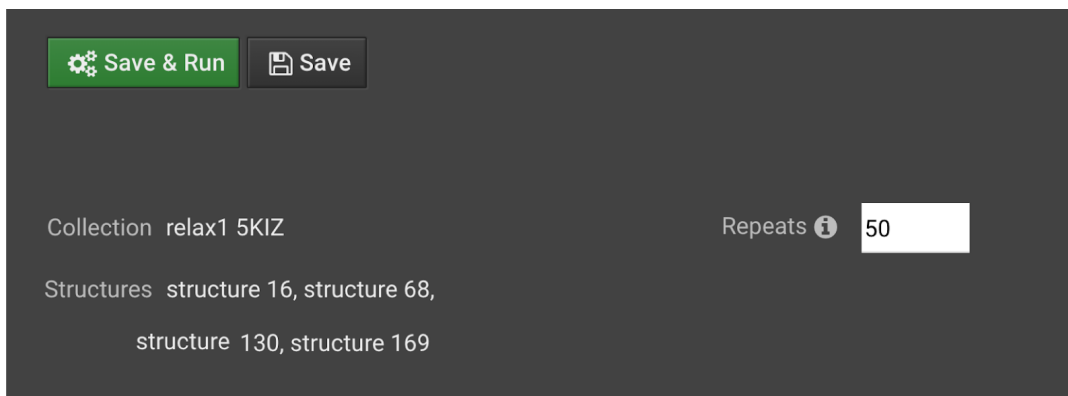
Enter [Repeats ⓘ 50 ⇕] which will run Relax 50 times for each of the 4 structures, totalling 200 Relax runs. Click [⚙ Save & Run] .

## 3) Analyze Second Round of Relax

Once the second Relax has run, the results will appear in the left window. Change the results file name to *relax2 5KIZ*. Click on the folder in order to analyze the results.
As described in Section 1 the structures generated from Relax are expected to have lower energy scores and the range of energy scores will narrow. Select the lowest energy structure and run another round of Relax with 5 repeats. Keep doing this with the lowest energy structure until there is no change in the generated structure's score..

Why are multiple rounds of Relax necessary?

Running the Relax method is a way to make aggressive changes to the protein's conformation in order to find new conformations that are more favorable. Many structural changes are sampled during Relax in order to find ways to improve the structure. Each round of Relax will always sample the same number of conformations. Sometimes, this is not enough sampling to find the lowest score, so more conformations must be sampled in order to find the best one.

In this case the results of an initial Relax run are used as a starting structure to run more Relax repeats. If Relax cannot find a better conformation, it will give you a Relaxed conformation with essentially the same score as the starting structure. This is explained further in the flowchart on the next page:
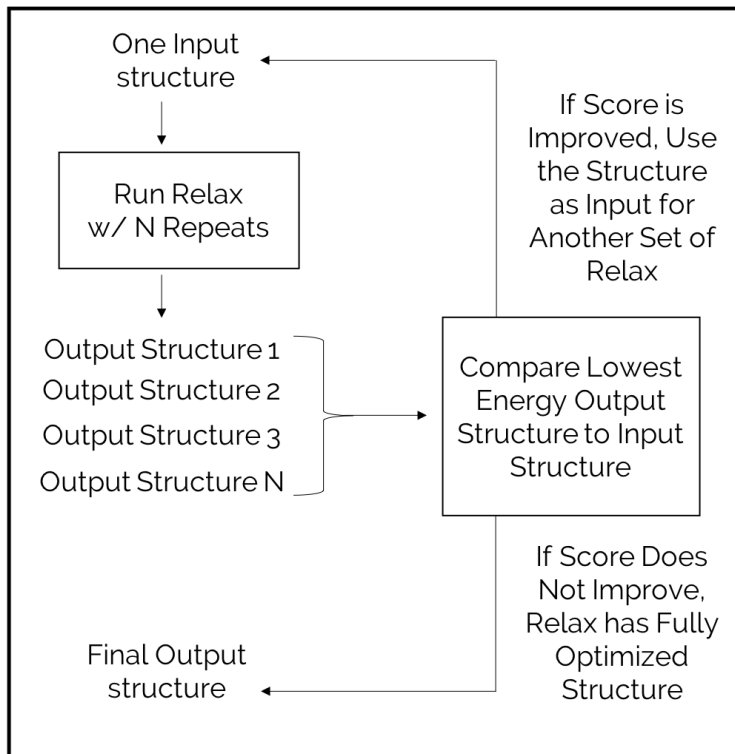
4.) Optimizing the Lowest Energy Structure
 Once Relax has generated the lowest energy structure, this final structure should be optimized with the Minimize method. For more information on Minimize, click here.

To run Minimize select the lowest energy structure generated from Relax and click Minimize ,
then ⚙ Run . Minimize will always have only 1 final structure, so you do not have to make repeats. Change the results file name to Optimized 5KIZ. This may have no score improvement, but it creates a folder with the one structure present. This can make a project easier to organize.