Tutorial Four: Increased Affinity to a Peptide using Relax Design, Sequence Logo

Estimated time: 30 minutes

This tutorial will instruct users how to use Relax Design to increase the affinity between two peptides, including how to assess whether affinity has increased. The Sequence Logo tool will also be introduced.

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

(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) Run Relax & Minimize

For this tutorial the structure 1OJ5 will be used to model how Design can be used to increase peptide affinity. First the 1OJ5 structure will undergo Relax and Minimize to find a starting structure with optimized energy.

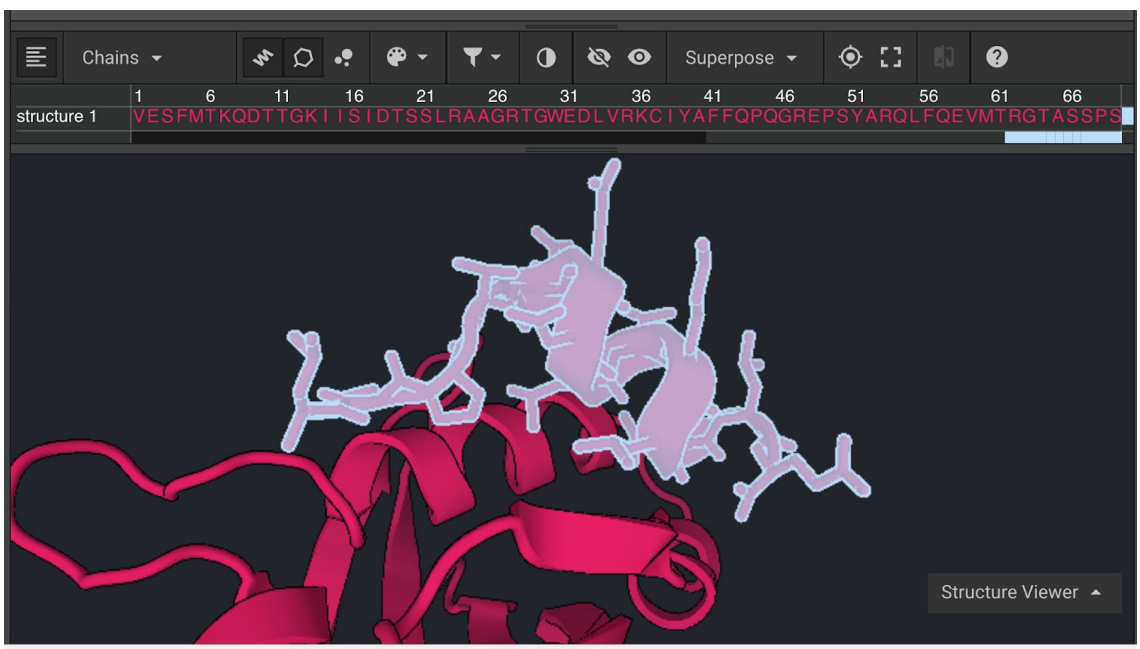
Select a Project or Create a Project where you will run this tutorial. Load structure 1OJ5. Run Relax, each round with 5 repeats, until the lowest energy conformation has been reached.

Minimize this final energy structure and rename the file *MinRelaxed 1OJ5*. This process of Relax rounds and Minimize is described in detail in Tutorial Two.

2) Design Increased Affinity Between Protein and Peptide

To increase the affinity between the two peptide chains in the 1OJ5 structure, residues involved in protein-protein interactions will be mutated. Using Relax Design, alternate amino acids will be sampled at selected residues to increase the affinity between the two peptide chains.

Click on *MinRelaxed 1OJ5* and click the in order to visualize the protein in the Structure Viewer. One way to zoom in on the region of interest is to switch the selection mode from Residues, which is the default, to Chains. Then click on the small peptide and click in order to center it. While the peptide is still selected, click in order to show sticks as demonstrated below.



Switch the selection mode back to Residues and select the residues whose side chains are oriented towards the protein-peptide interface. It is important to note that prolines should not be selected for mutations because it is usually disruptive to mutate away from prolines. Click

in order to show the sequences and highlight residues 106, 113, 116, and 117 (hold down command or control while selecting).

Click and change the color in order to make it easier to see that these residues are important to binding. Create a Selector for the peptide selection by clicking Create, then clicking to change the name to Peptide. Click the to the left of the structure name in order to select it.

To sample alternate amino acids for the selected residues in the peptide click Relax Design in the right window. Choose Selector Peptide and enter Repeats 5. Then highlight all possible mutations by clicking Pos. in the top left corner of the mutation table as shown below.

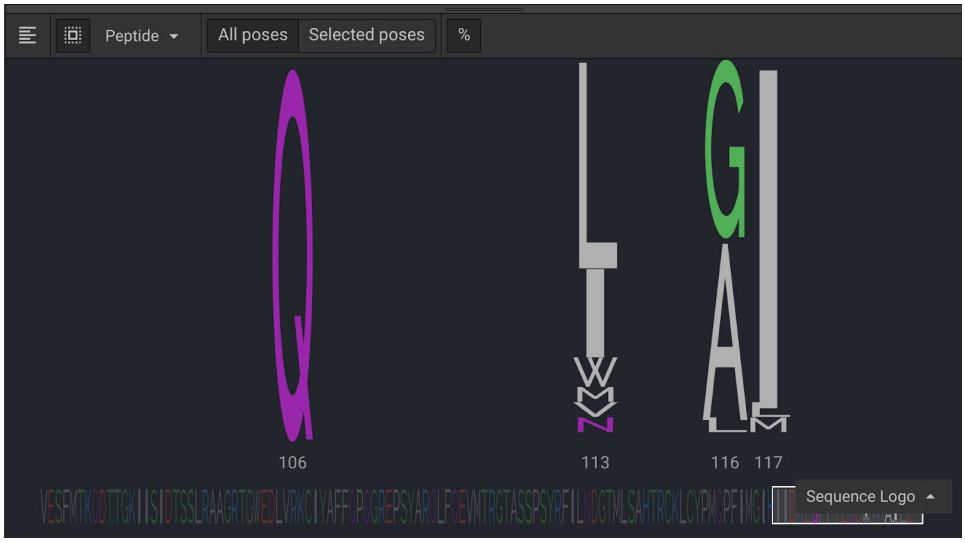
Design mutations

Download mutations | Upload mutations from CSV | Upload mutations from PSSM

Pos.	Original Residues	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
106	L	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
113	L	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
116	L	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
117	L	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V

Click Save & Run. Once the Design has run change the name of the Design run to *relD of 1OJ5 and* click on the folder in order to bring the results into the center window.

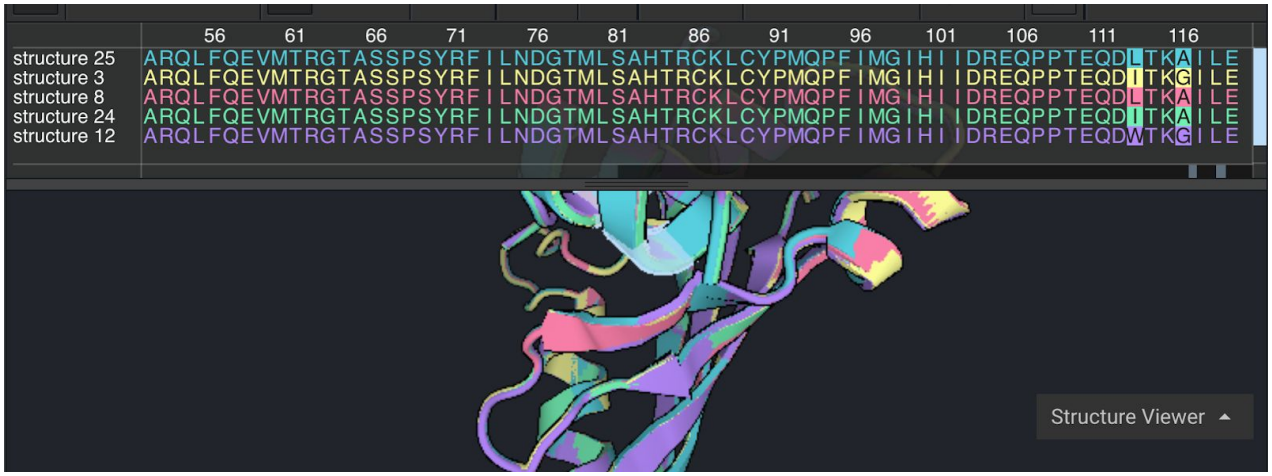
Click Structure Viewer in the lower center window and change the button to Sequence Logo which will display which mutations were favorable. Click the on the top left of the Sequence Logo window and click the arrow to the right ot it to select the Peptide selector. This will hide all the residues that were not part of the selector. Scroll to the right to see the four residues in the selector as shown below.



The original sequence for these four positions was LLLL. You can see that L was only favorable at position 113. Position 106 preferred Q in all of the simulations. Positions 116 and 117 favored GA and I respectively. Results above are from a larger number of repeats in order to show diversity.

To look at the structure to see why these mutations were preferred switch Sequence Logo back to Structure Viewer. Now rank the structures by Score and click only the for the top five structures.

Click in order to show their sequences. Then click in order to highlight the sequence differences. Scroll over to the right to see them.



In this example there were only two positions, 113 and 116 as highlighted in the sequences above, that differed across the top five structures. As suggested in the sequence logo, residue 113 would be most stable as an isoleucine or leucine and residue 116 as alanine or glycine.

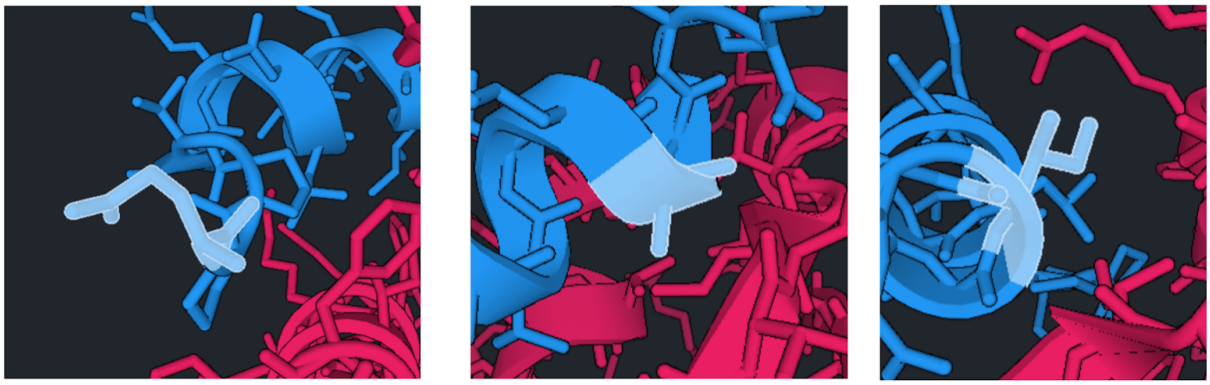
The other two new mutations which were introduced at (Q106 and I117) are present across the top five structures suggesting they are best mutations for those positions.

Viewing and Comparing Potential Mutations

The mutations which were generated with Relax Design can be viewed and visually compared to the starting residues. The lowest energy structure from the Relax Design in this example is structure 25. Deselect the for the four structures and select to show structure 25.

Click on the whole structure and click in order to show sticks. Select residues 106 to 119 by showing the sequence via and clicking on the residues. Then click and change the color in order to make it easier to see that these residues. Shown below are the three positions that were mutated in the structure.

Three mutation positions with the most-preferred mutant

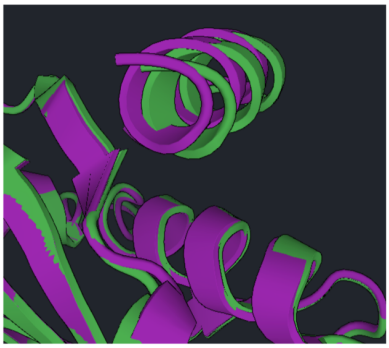


L106Q L116A L117I

With the L106Q mutation this side chain points away from the structure so it may be favorable because of improvement in solvation.

For L116A, the smaller side chain allows the peptide to shift towards the protein's beta sheet (see image to the right). The other preferred mutation at this site, glycine, also had a

Mutant (purple), WT (green)



smaller side chain to possibly allow the peptides to shift closer. This may not have been sterically possible before.

For L117I, the isoleucine packs into the gap which may have improved packing compared to leucine did.

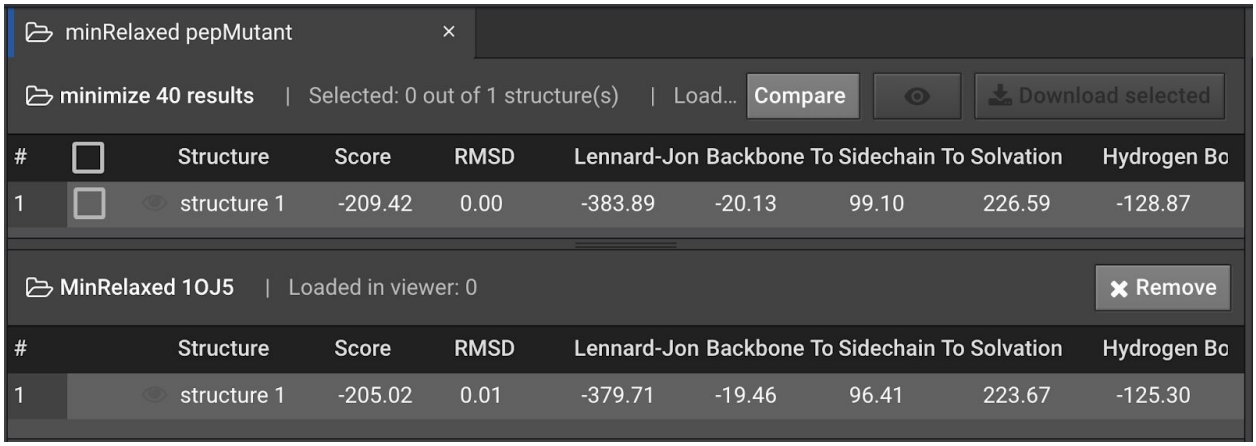
In order to create an alignment of a specific region between two structures like the image in purple and green on the previous page, click Compare. In the drop down window, select the relaxed (*relD of 1OJ5)* and minimized wild type structure (MinRelaxed 1OJ5).

Ensure that the mutant and wild type structures are two different colors and align everything except the peptide. Do this by selecting residues 1 to 105 for both proteins. Click Superpose to open a drop down and click Selected backbone atoms.

As long as you leave the peptide out of the selection, this will align the protein so you can see how the peptide shifted when the mutations were modeled. You may want to go into full screen mode by clicking in order to visualize the differences better.

Using Relax and Minimize to Compare Mutations

In order to verify that the mutations are energetically favorable first Relax and Minimize the structure generated from Design as follows. Click the to the left of the structure name and run a Relax with 5 repeats. Then run a Minimize on the most energetically favorable structure from Relax. Compare the energy of the Relaxed and Minimized mutant with the Relaxed and Minimized wild type as shown below:



In the example above, the energy improved by 4.4 REUs from the minimized 1OJ5 structure to the minimized Design result. This is a significant difference for only 3 point mutations. However without testing it is not clear that the mutations have improved affinity. The mutations with lower energy structures may have improved stability within the peptide which doesn't affect affinity.

Predicting Peptide Stability Versus Peptide Affinity

One way to computationally predict whether the mutations are favorable due to peptide stability is to model the peptide without the protein. You will need to create and download a structure for 1OJ5 with only the smaller peptide chain present.

You can manually delete selections of the protein in a text editor or you can use a visualization program such as Pymol. The manipulated file needs to be a .pdb file with only the peptide residues present.

To download the original PDB file click the 1OJ5 folder to bring it into the center window. Click the ☑ to the left of the structure name in order to select it. Then click Download selected to open a drop down window that allows you to select pdb, fasta, or scores. Select PDB files. Once only the smaller peptide chain residues remain the PDB file should look like this:

Peptide.pdb

```
ATOM   1677  N   LEU B 106       9.780  13.464  20.414  1.00  0.00           N
ATOM   1678  CA  LEU B 106       9.540  14.311  21.629  1.00  0.00           C
ATOM   1679  C   LEU B 106      10.845  14.936  22.141  1.00  0.00           C
ATOM   1680  O   LEU B 106      11.733  14.228  22.632  1.00  0.00           O
ATOM   1681  CB  LEU B 106       8.882  13.448  22.719  1.00  0.00           C
ATOM   1682  CG  LEU B 106       8.499  14.104  24.050  1.00  0.00           C
ATOM   1683  CD1 LEU B 106       7.448  15.179  23.863  1.00  0.00           C
ATOM   1684  CD2 LEU B 106       7.983  13.011  24.992  1.00  0.00           C
ATOM   1685 1H   LEU B 106       8.914  13.071  20.104  1.00  0.00           H
ATOM   1686 2H   LEU B 106      10.169  14.028  19.686  1.00  0.00           H
ATOM   1687 3H   LEU B 106      10.417  12.727  20.641  1.00  0.00           H
ATOM   1688  HA  LEU B 106       8.867  15.123  21.357  1.00  0.00           H
ATOM   1689 1HB  LEU B 106       7.963  13.023  22.318  1.00  0.00           H
ATOM   1690 2HB  LEU B 106       9.557  12.630  22.970  1.00  0.00           H
ATOM   1691  HG  LEU B 106       9.375  14.588  24.483  1.00  0.00           H
ATOM   1692 1HD1 LEU B 106       7.203  15.620  24.829  1.00  0.00           H
ATOM   1693 2HD1 LEU B 106       7.833  15.953  23.199  1.00  0.00           H
ATOM   1694 3HD1 LEU B 106       6.551  14.739  23.428  1.00  0.00           H
ATOM   1695 1HD2 LEU B 106       7.705  13.456  25.948  1.00  0.00           H
ATOM   1696 2HD2 LEU B 106       7.111  12.530  24.548  1.00  0.00           H
ATOM   1697 3HD2 LEU B 106       8.765  12.269  25.151  1.00  0.00           H
ATOM   1698  N   PRO B 107      11.004  16.249  22.013  1.00  0.00           N
ATOM   1699  CA  PRO B 107      12.223  16.872  22.517  1.00  0.00           C
ATOM   1700  C   PRO B 107      12.396  16.717  24.041  1.00  0.00           C
```

Load this new structure into CAD as a new project. Create the three point mutations by running a single Design run, noting that the position numbers have been renumbered. You do not need to create a Selector for this. You can select the mutations manually in the mutation chart of the Design tab. Create these mutations:

Pos.	Original Residues	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
1	L	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
2	P	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
3	P	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
4	T	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
5	E	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
6	Q	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
7	D	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
8	L	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
9	T	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
10	K	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
11	L	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
12	L	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
13	L	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V
14	E	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V

Leave the number of repeats as 1 and click Save & Run. Rename the folder Peptide Mutant.

The WT Peptide and this new Peptide Mutant should be Relaxed and Minimized before comparing their energies. Since the peptide is so small, running 5 Relax repeats is more than enough for both the WT and the new mutant. Then run Minimize on the one with the best energy for both the WT and the new mutant. Using these, click Compare to see the energy difference.

minRelaxed mutant peptide ×

minimize 44 results | Selected: 0 out of 1 structure(s) | Load... Compare Download selected

#	Structure	Score	RMSD	Lennard-Jon	Backbone To Sidechain	To Solvation	Hydrogen Bo
1	structure 1	-16.23	0.00	-32.61	-3.15	11.39	22.80	-10.26

minRelaxed WT peptide | Loaded in viewer: 0 Remove

#	Structure	Score	RMSD	Lennard-Jon	Backbone To Sidechain	To Solvation	Hydrogen Bo
1	structure 1	-15.76	0.00	-32.58	-3.18	9.91	22.32	-9.34

In this case, the energy improved by 0.5 REUs, suggesting that the mutants are more likely to affect affinity to the protein than inner stability.