Introduction to the Schrodinger Bioluminate package

Session 2: Prime

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Learning goals:

By the end of this workshop, participants will be able to:

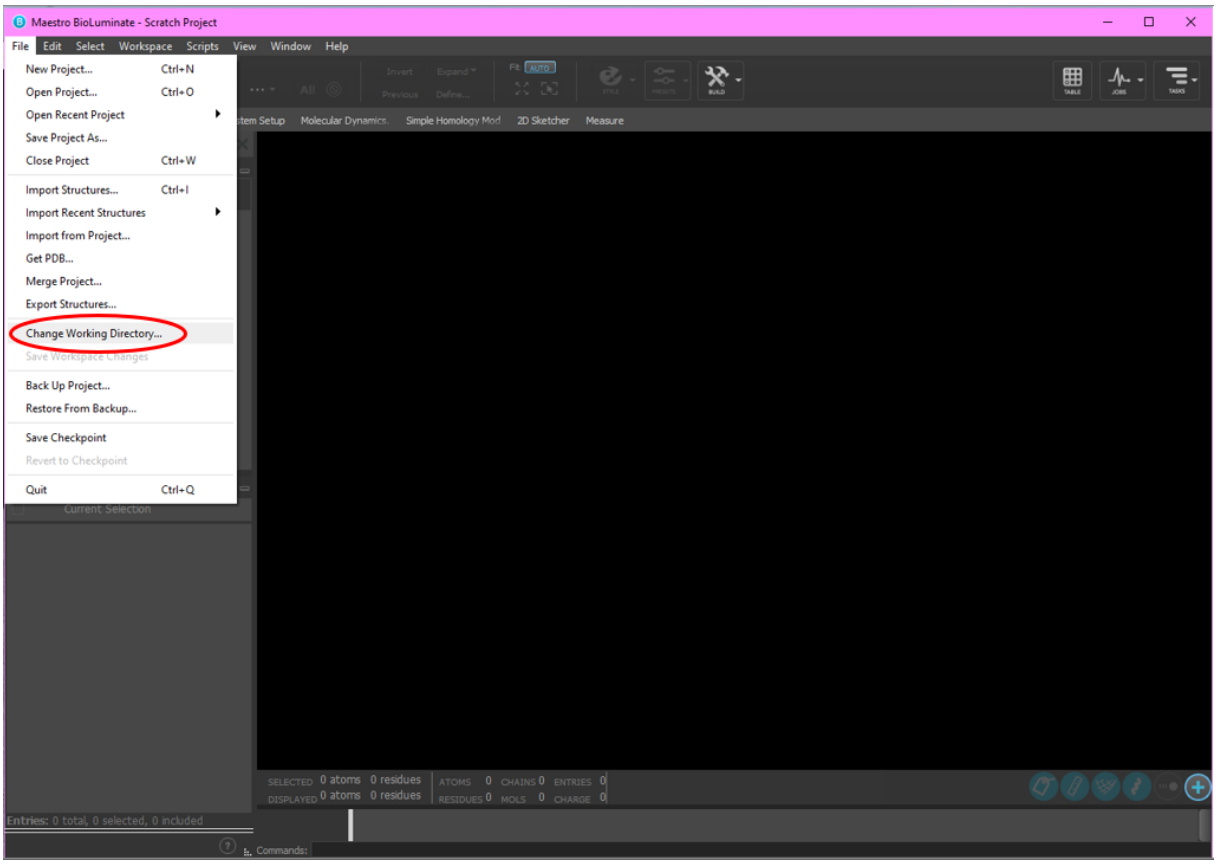
- Know where to find additional resources for homology modelling.
- Know where to find and how to use the commands used in modelling proteins
- Independently model a protein from a protein sequence.
- Assess the quality of a homology model.

Contents:

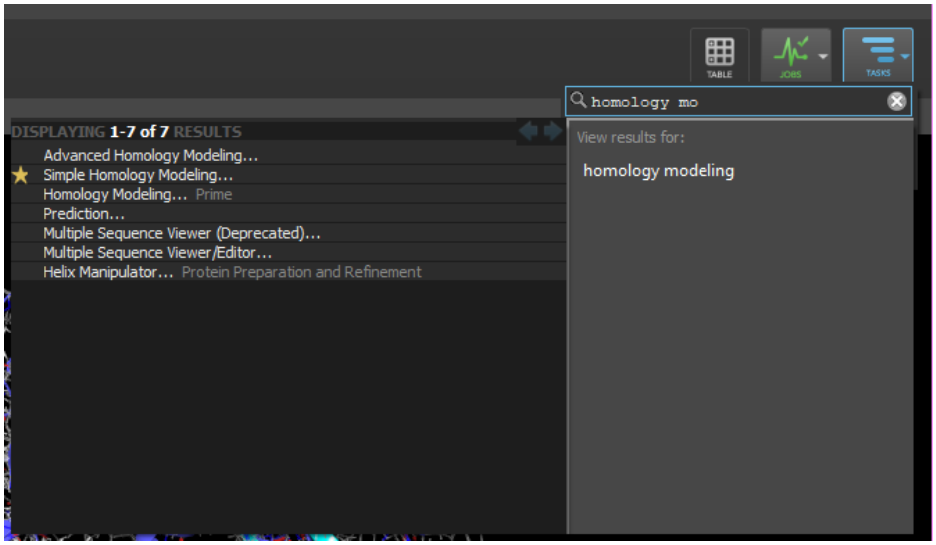
1. Intro to Prime
2. Schrodinger resources: Where to find them and how to use them
3. Learn the Maestro interface:
    a. Demonstration of importing pdbs + user interface
    b. Try it yourselves
    c. Demonstration of protein preparation & analysis
    d. Try it yourselves
4. Evaluation

Getting started with Prime

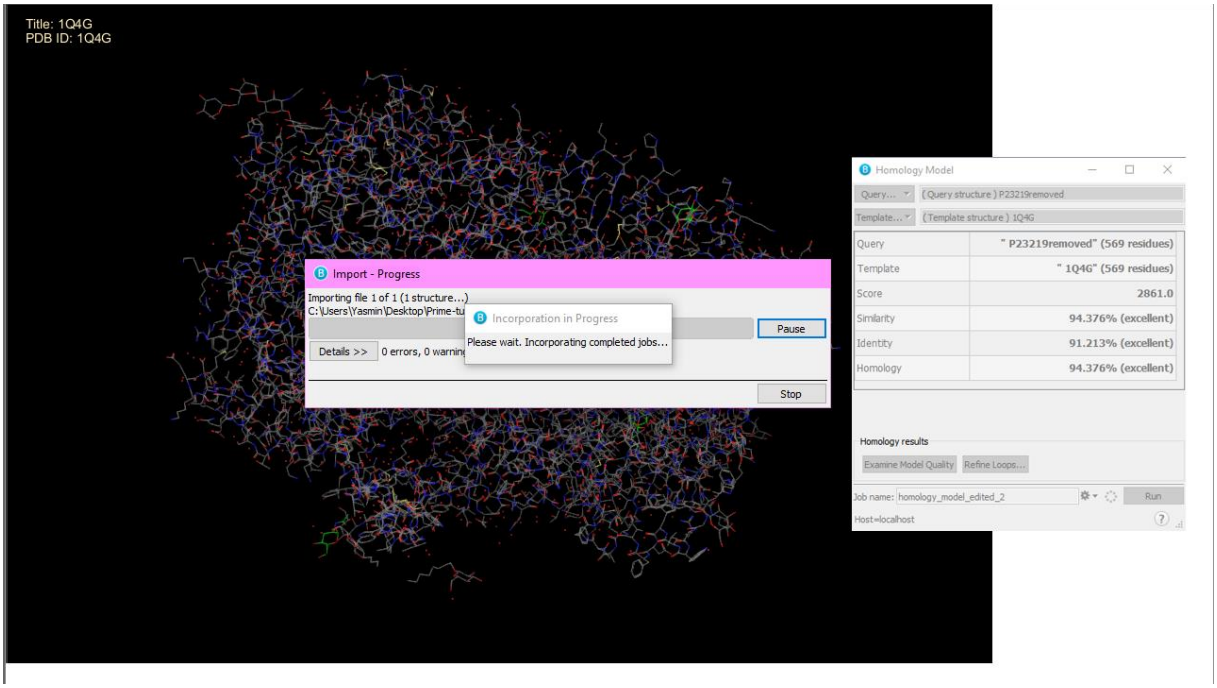
1. Download the Prime directory from Slack or copy it from Google Drive (G:\Shared drives\Chemistry Boxer Lab - Current Members\General BoxerLab Folders\Schrodinger_Workshop).
2. Put the Prime directory on your desktop.
3. Change the working directory to your Prime directory on the desktop:

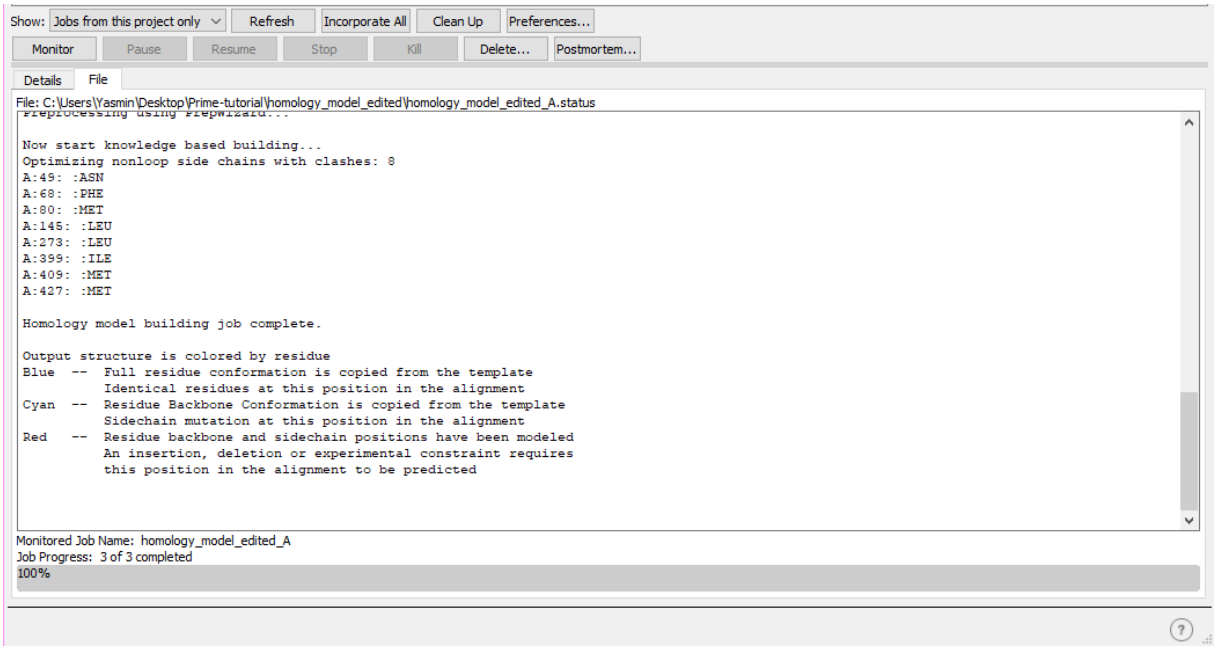


4. Go to tasks and see which options you have for homology modelling. Choose the Simple Homology Modelling for now.



5. Today, you will create homology models of human COX-1 based on the crystal structure of bovine COX-1 used in the previous workshop (PDB ID: 1Q4G). All the files you need are in the downloaded Prime folder.
    a. Start by choosing the edited sequence as your Query and the 1q4g structure for your Template.
    b. Click run.
    c. Choose the B chain.
    d. Wait 2 min for the structure to be created and incorporated into the workspace.





Analysis

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Output structure is colored by residue
Blue   --   Full residue conformation is copied from the template
            Identical residues at this position in the alignment
Cyan   --   Residue Backbone Conformation is copied from the template
            Sidechain mutation at this position in the alignment
Red    --   Residue backbone and sidechain positions have been modeled
            An insertion, deletion or experimental constraint requires
            this position in the alignment to be predicted
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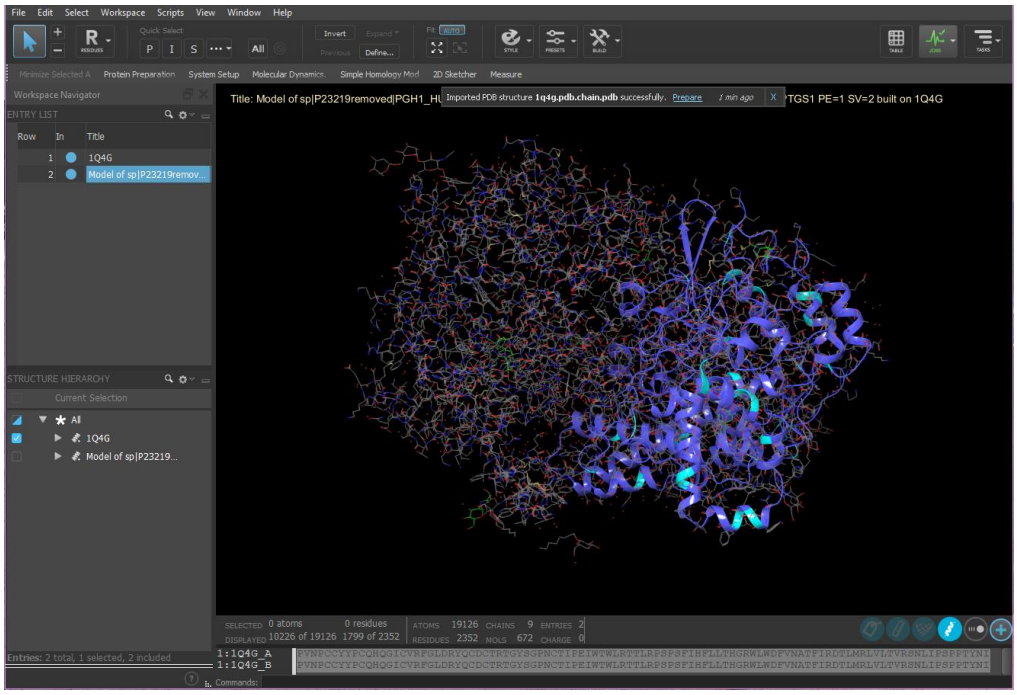
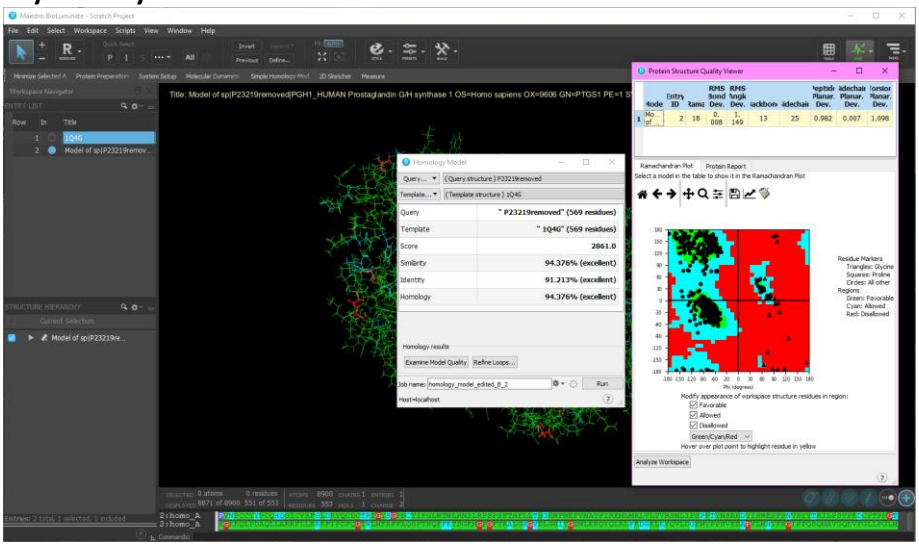


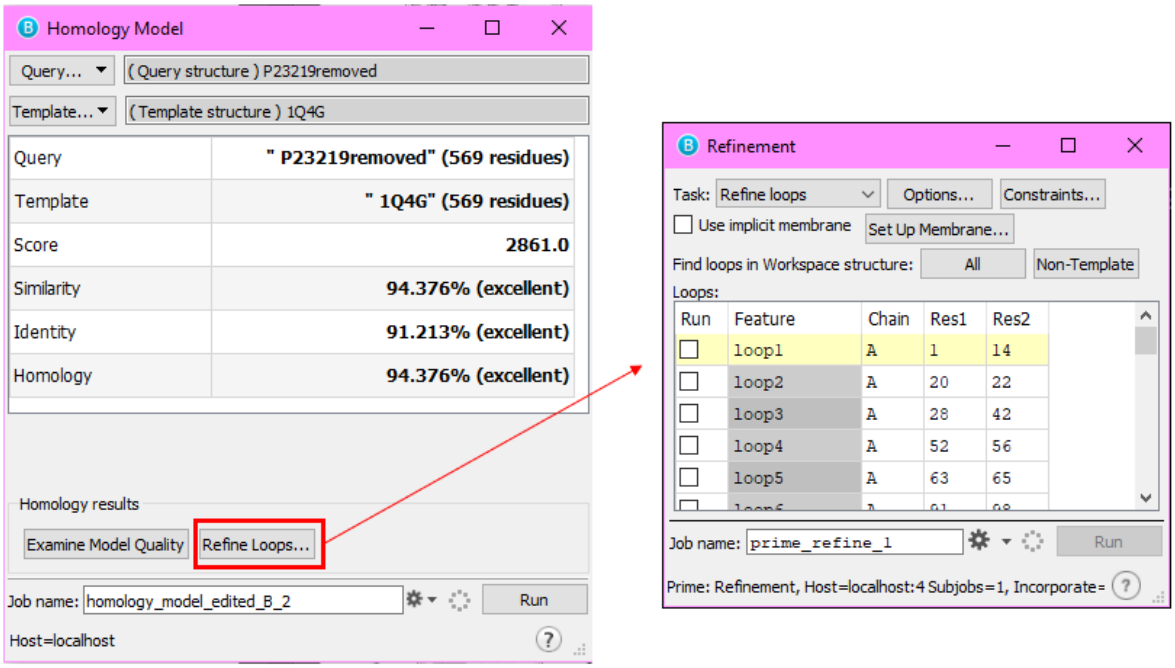
How well did your homology model match the template?

1. In the homology model windows – Click Examine Model Quality. This brings up a Ramachandran plot and colors the amino acids according to which area they are in.

Are there any non-Glycine residues in the disallowed areas?



2. If you want to refine the structure further you can go to Refine Loops, but as this can be a time-consuming process, we will not do it today.



Well done on creating your first homology model! Now try to do it on your own using the non-edited sequence.

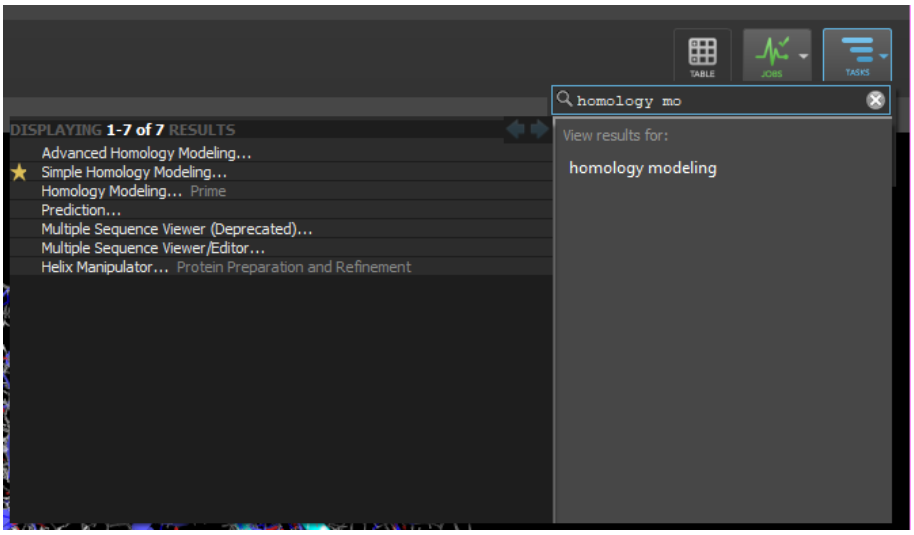
Questions to answer after finishing homology modelling of the non-edited sequence:

1. Why did you get this result? Discuss with your partner.
2. Considering the sequence identity, was it expected?
3. What was done in the edited sequence to improve the model?

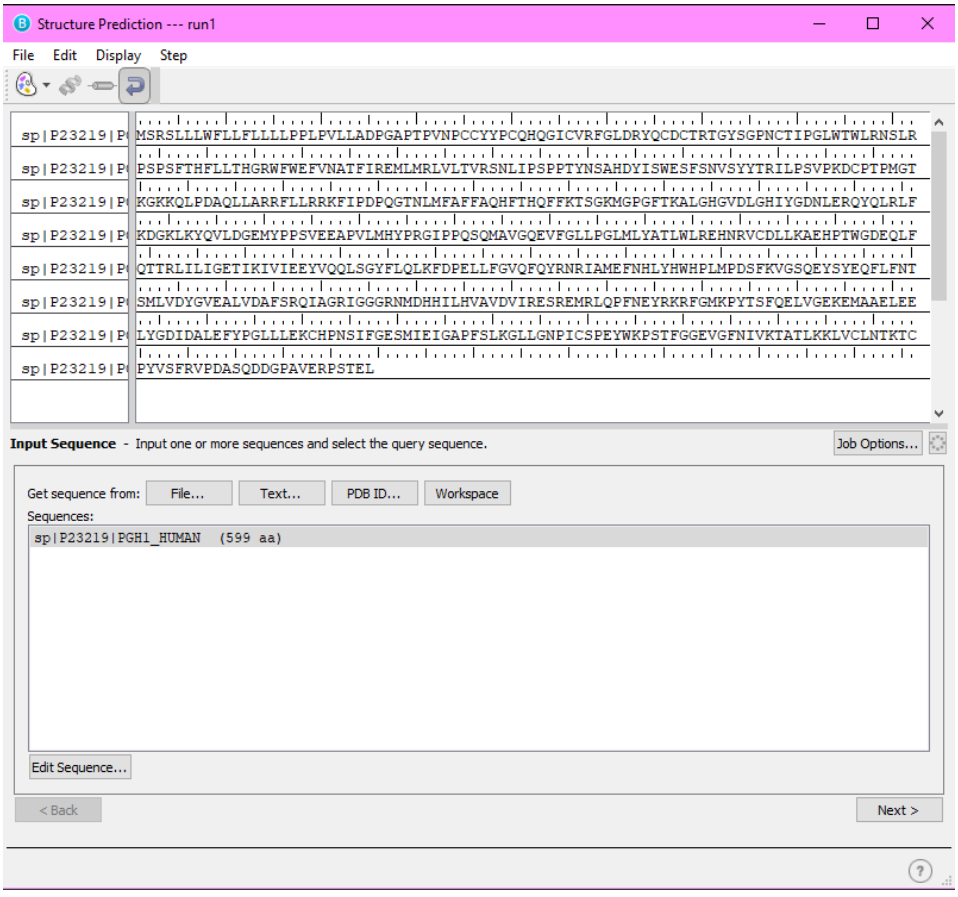
Advanced modelling

To fix the problems observed in the last homology modelling we will use the advanced tools in Prime.

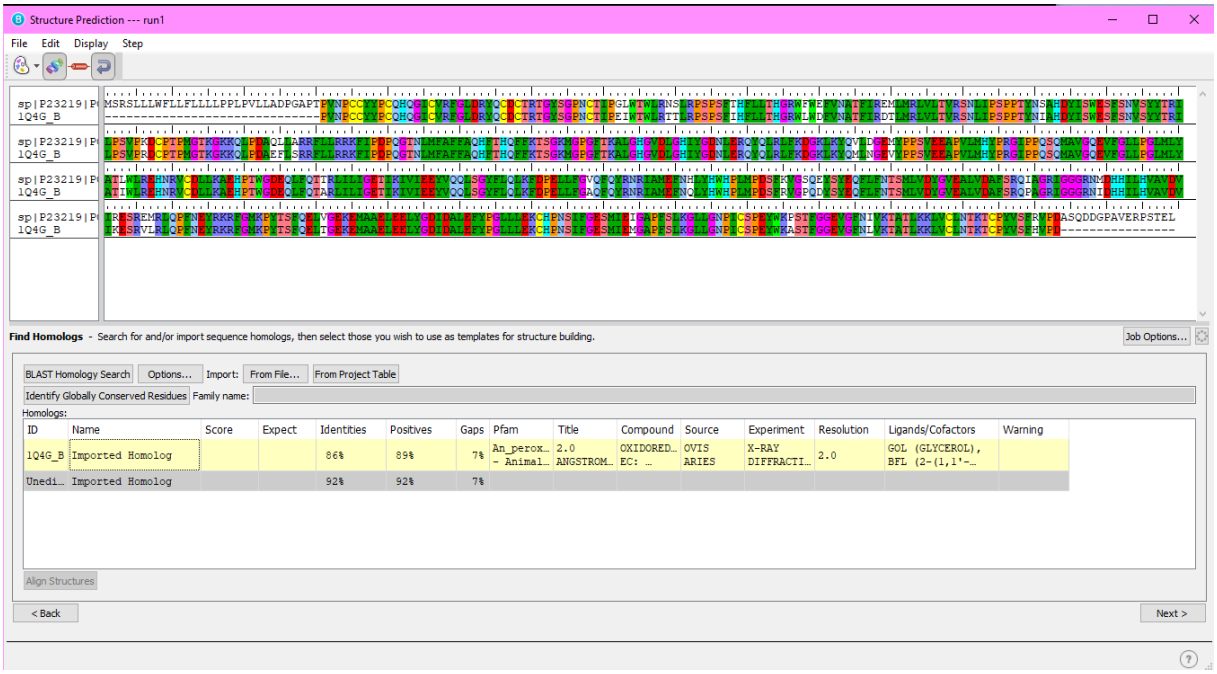
1. In the menu, choose Homology Modeling Prime (The Advanced Homology Modelling option opens the same window)



2. Choose the non-edited sequence as your **Input sequence** (File …). Click Next > .



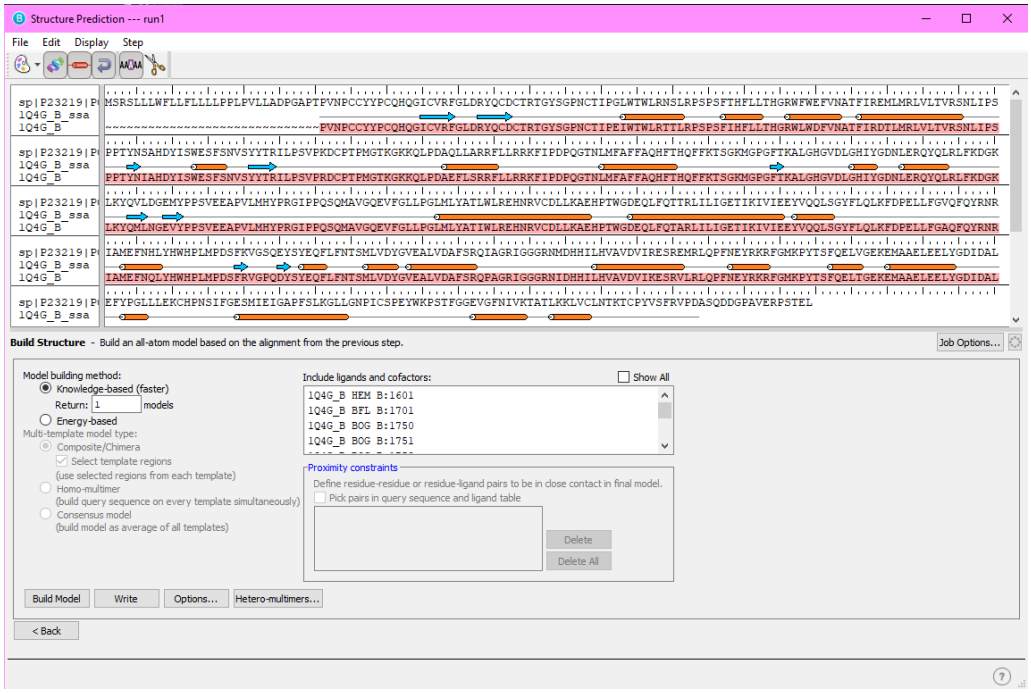
3. Choose the 1q4g.pdb file, either by Import File… or Import From Project Table (don't forget to highlight the correct file in the Workspace Entry list Project Table before selecting this option).



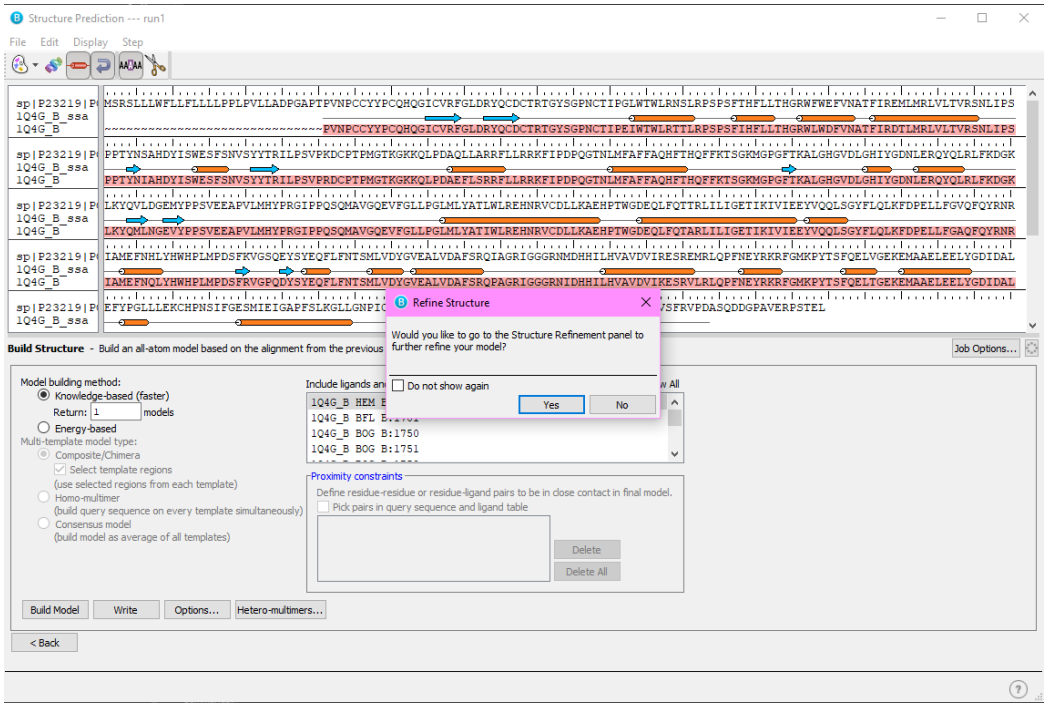
How do you think that this method is different from the Simple homology modelling, based on what you see in this window? How do you think the results will differ? Discuss with your partner.

4. Explore the different option at this step. What do the different buttons under the main menu do? When done, continue by clicking Next.
5. In this window you can choose alignment method. For now, keep the ClustalW and click Next.

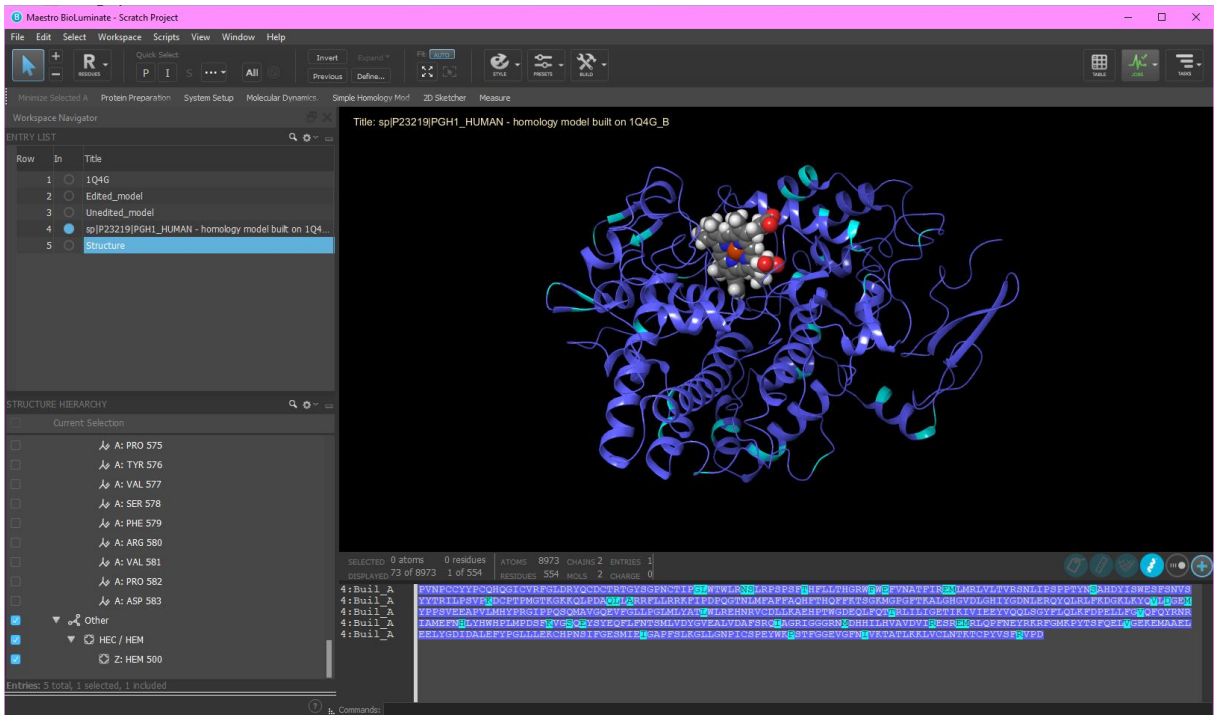
6. In this step you can choose to import additional structures from your template structure. Keep the HEM group and look through the different options for building your model. To save time here, use the knowledge-based method and return 1 model. Click Build Model to generate the new homology model. This process will take at least 5 min, so now is the time to stretch your legs and get a small snack!



7. When the model has completed you will get the option of refining your loops. Let's skip this step!



8. Analyze your new structure. Is it a good model? Why? Is it any different from your very first model?

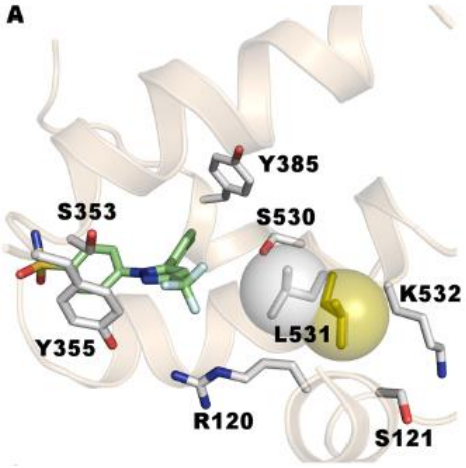


9. Save your model using an appropriate name. You will use it again in the docking workshop.

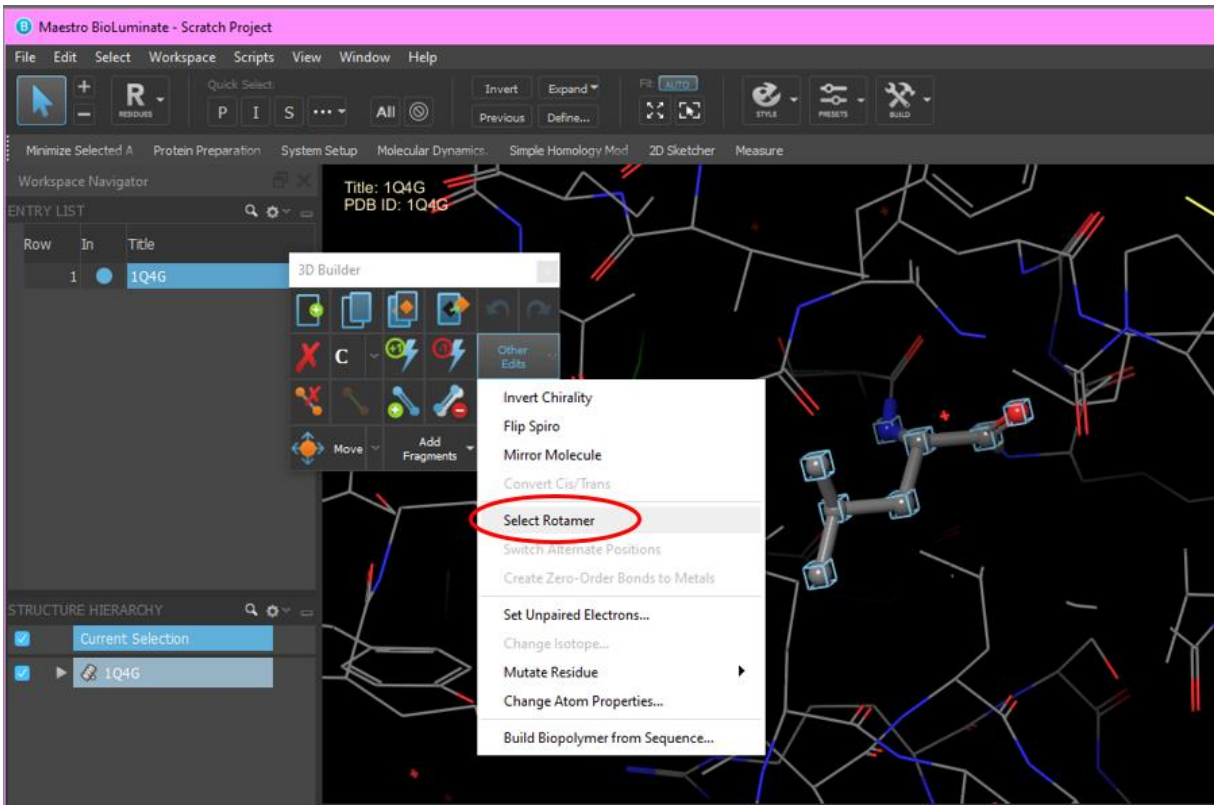
Tweaking your model

In this section we will edit the protein in two ways. First, we will rotate a residue and save the new structure. Next, we will mutate a residue and see how it changes the binding pocket.

It has been demonstrated that Leu531 can exist in at least two rotational conformations, the native "closed" (grey) and an induced "open" (gold) conformation.



1. Model the residue into the open conformation by selecting it and choosing 3D Builder – Other Edits – Select Rotamer



2. Did you choose the same rotamer as your partner? Export the new structure under a new name, easily distinguishable from the previous structure. You will use this structure in the molecular docking session too.

It has been suggested that selectivity of coxibs, second-generation NSAIDs is due to a mutation in the gate-keeping residue Ile523, which sits between the main binding pocket and a side-pocket that is occupied by these inhibitors.

3. Mutate Ile523 to Val523 using the Mutate Residue option in the Build menu. Export the structure.

By the end of this session, you should have at least three properly named homology models in your main Prime folder. Create a new folder and move these files to it.