## Constraining atom in transition state calculation protocol for sphingomyelin synthase-1 enzyme

1. To begin, click the **GUIDE** menu on the YASARA window taskbar and select the **QM calculation** option.
2. In the QM calculation window, enter the working directory information and select **ORCA** as the calculation method. Then, click **OK**.
3. A new window will appear to select the **Type of QM calculation**. Select the **Constraining QM** option and click **OK**.
4. Next, choose the functional and basis set information.
5. After selecting the functional and basis set information, load the sphingomyelin synthase-1 (SMS1) and phosphatidylcholine complex structure (***SMS1-PC_R.yob*** file) on the YASARA window, and click the **Continue** button.
6. To increase the number of climbing images in the transition state calculation, the user can increase the number of images, which is set to 8 by default.
7. Select the atoms (QM1) for the quantum mechanical (QM) calculation from the structure and click the **Continue** button.
8. The next step is to select atoms for constraining during the QM calculation. Select the desired atoms along with the QM1 atoms from the YASARA window and click the **Continue** button.
9. The plugin will automatically compute the charge and initiate the calculation. The remaining steps are the same as the **Transition state** calculation using the GUIDE.

Please watch the video at the following link for more information. https://github.com/YAMACS-SML/GUIDE/tree/main/video_tutorials/2_GUIDE_transition_state_calculation.mp4