**Gaussian protocol**

The following describes how to use GaussView to build amb peptides and set up calculations to submit to Gaussian09 to geometry-optimize the starting structures.

1. Login to the **GaussView** program.
2. In the **GaussView** window, click on **View** and **Builder** and select the **Biological Fragments** button.
3. From the **Biological Fragments** menu, choose **Amino-Terminal Fragment** and **Histidine 2 (Hie)**
4. Go to the new window and click on the empty space in the new window. The histidine fragment will appear.
5. Go back to the **Biological Fragments** menu and choose **Central Fragment** and select **Cysteine 1**
6. Go to the new window and join the two amino acids by clicking on the C-terminal end free bond of histidine.
7. Continue this process to build the amino acid sequence of the amb peptide.
8. For the C-terminus amino acid, in the **Biological Fragments** menu, select **Carboxyl-Terminal Fragment** and **Cysteine 1**.
9. To acetylate the amino-terminal, from the **Builder** window select the **Element Fragments** button twice. This will bring up the **periodic table** window.
10. Choose the carbon atom from the **periodic table** window and **Carbon Trivalent**.
11. Go to the new window and click on one of the hydrogen atoms at the amino-terminal end of the amb. The trivalent carbon will substitute for the hydrogen atom.
12. Choose the oxygen atom from the **periodic table** window and **Oxygen Trivalent**.
13. Go to the new window and click on the end of the carbon double bond to add the oxygen to build the acetylation group.
14. From the **periodic table** window, select carbon and the **carbon tetrahedral** option.
15. Go to the new window and click on the hydrogen connected to the carbon single bond to add the methyl group to build the acetylation group.
16. After the desired sequence is achieved, you will have to modify its conformational structure to substituent groups into positions where they can chelate the Cu(I) or Cu(II) ions. To do this, you will need to use the following functions.
17. Right-click on the structure and select **Builder** and **Modify** **Dihedral**
18. Select 4 atoms in a row to change the dihedral angle, and select which atoms or groups are to be fixed or rotated. Change the value of the dihedral until the desired configuration is achieved, and click on **OK**.
19. To modify bond angles, right-click on the structure and select **Builder** and **Modify** **Angle**
20. Select 3 atoms in a row and select which atoms or groups are to be fixed, translated, or rotated. Change the value of the angle until the desired configuration is achieved, and click on **OK**.
21. To modify bond lengths or the type of bond, right-click on the structure and select **Builder** and **Modify** **Bond**.
22. Select 2 atoms in a row and select which atoms are to be fixed or translated. Change the value of the bond distance until the desired bond length is achieved, and click on **OK**.

NOTE: During the manipulation of the conformational structure conserve the valence nature of the bonds the *trans*-peptide bonds.

1. When the substituent groups are in the desired positions. Choose the copper atom from the **periodic** **table** window and **Copper Atom**.
2. Go to the new window and click on the space in the molecule you want the copper to be added.

**Calculation Setup**

1. When the amb complex is in the desired configuration, set up the calculation as follows.
2. Right-click on the structure and select **Calculate** and **Gaussian Calculation Setup**.
3. In the calculation setup window, select the **Job Type** from the menu bar and choose **Opt+Freq** and select the options: **Optimize to a Minimum**, and **Calculate Force Constants Once**.
4. Under the **Method** tab, select the type of calculation to be performed to **Ground State**, **Semi-empirical**, **Default Spin,** and **PM6**.
5. Specify the net charge of the molecule, e.g., -1.
6. Specify the multiplicity of the molecule according to the rule S+1 where S is the number of unpaired electrons, e.g., Zn(II) has no unpaired electron, Multiplicity = 1. Ni(II) has two unpaired electrons, Multiplicity = 3.
7. Right-click on the new window showing the molecule and select **File** and **Save**.
8. To submit the job to Gaussian09, consult your computer system administrator.