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 were 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, select which atoms or groups are to be fixed or rotated. Change the value of the dihedral until the desired configuration is achieved, click 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 angel until the desired configuration is achieved, click 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, click 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 positons. 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, setup the calculation as follows.
2. Right click on the structure and select “Calculate” and “Gaussian Calculation Setup”.
3. In the Calculation set up 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”, “DFT”, “Default Spin” and “B3LYP”. Select the Basis set to “LanL2DZ”
5. Specify the net charge of the molecule, e.g., 0, 1, -1, -2.
6. Specify the multiplicity of the molecule according to the rule S+1 where S is the number of unpaired electrons, e.g., Cu(II) has one unpaired electron, Multiplicity = 2.
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.