

CHEM 463: Advanced Inorganic Chemistry Modeling Metalloproteins for Structural Analysis

Purpose: The purpose of this laboratory is to introduce some of the basic visualization and modeling tools for viewing three dimensional structures of metalloproteins discussed in lecture. The student will become familiar with local and web-based resources for protein structural analysis.

Background:

Overview of Metals in Biology

Bioinorganic chemistry exists at the interface of classical inorganic chemistry and biology. Although the biology of organisms is focused on organic chemistry, inorganic elements are essential to many life processes. Table 1 below lists some of the essential inorganic elements and their roles in biological systems.

<i>Metal</i>	<i>Function</i>
Na, K	Charge carrier; osmotic balance
Mg, Ca	Structure; hydrolase; isomerase
V	Nitrogen fixation; oxidase
Mo	Nitrogen fixation; oxidase; oxo transfer
W	Dehydrogenase
Mn	Photosynthesis; oxidase
Fe	Dioxygen transport; oxidase; electron transfer
Co	Oxidase; alkyl group transfer
Ni	Hydrogenase; hydrolase
Cu	Oxidase; dioxygen transport; electron transfer
Zn	Structure; hydrolase

Table 1: Some Properties of 3d Transition Metals

In biological systems, metals are found as constituents of proteins. The use of metals in proteins can be broadly separated into four classes:

- A. *Transport:* Metalloproteins in this category are responsible for molecular transport. The most obvious examples of this class of metalloproteins are the oxygen transport systems used in respiration including myoglobin-hemoglobin family, hemocyanins and hemerythrins.

- B. *Electron Transport:* Unlike carbon, many metals have other stable oxidation states that are energetically accessible at physiological conditions. These metals are ideal for the transport of electrons in processes that require oxidations or reductions to a substrate. Some examples of common electron transport metalloproteins include iron-sulfur clusters and cytochromes.

- C. *Structural Support*: Metal ions have the ability to access a wide variety of coordination states, many of which are different than the traditional sp^3 tetrahedral carbon center found in organic chemistry. In some cases, the metal simply defines the overall tertiary shape of the protein while in other cases the presence or the absence of metal ions and subsequent changes in protein configuration may be involved in protein/enzyme activity (metalloregulation). The well-known “zinc fingers” are an example of the first type of structural support that involve a Zn^{2+} ion which forms the central core of a wide variety transcription factor proteins. Iron-sensitive metalloproteins control the regulation and production of ferritin and other iron transport systems in mammals are examples of the second type of structural support.
- D. *Metalloenzymes*: Metalloenzymes are an important subclass of metalloproteins wherein the metal is active in some catalytic cycle in a biological system. Many of the transformations accomplished by metalloproteins involve small molecule substrates.

X-ray Structure Analysis of Metalloproteins

X-ray analysis is one of the most powerful tools for the assignment of absolute structure. X-ray's have a wavelength on the order of approximately 1 Å, which is the scale of interatomic distances in molecules. For small molecules, modern crystallographic techniques have the ability to provide resolution on the order of 0.01-0.001 Angstroms.

X-ray techniques can be applied to bioinorganic molecules and metalloproteins, but there are several complicated factors. First, the sequence of the metalloprotein must be known so that electron densities can be appropriately assigned to the correct residues. Second, crystals of the protein must be grown. Finally, heavy-atom derivatives must often be prepared to provide a suitable electron density map for solving the structure.

Domain Types & Structural Motifs

There are several different levels on which protein structure can be described. *Primary* structure refers to the local interatom connectivity. The covalent bonds determine the amino acid sequence. *Secondary* structure deals with local conformation over a small region or sub-unit. Secondary geometry is often controlled by hydrogen bonding, although in metalloproteins metals may play a crucial role in the organization. *Tertiary* structure describes the “folds” of a polypeptide chain in the context of the larger protein. The *quaternary* structure refers to the assembly of multiple polypeptide subunits into the complete protein.

There are three common secondary structures encountered: α -helix; parallel β -sheet; anti-parallel β -sheet. The α -helix is a tightly coiled rod-like structure that is typically short (ca 40 Å) with internuclei distances on the order of 1.5 Å. β -sheets are an extended sequence of amino acids where multiple polypeptide chains interact with each other.

Typical internuclei distances are on the order of 4 Å. The parallel/anti-parallel assignment tells if the chains are moving in the same direction or opposite directions.

Outline of Procedure:

For this laboratory, we will utilize the visualization tools contained on the PDB's website. The PDB is the repository for structural information for a wide variety of proteins. Data can be viewed on the website directly using available tools as well as downloaded for use on local systems.

In today's lab we will example four well-characterized metalloproteins that correspond to the four categories described in the background. The metalloproteins in today's lab are:

Transport Protein: Hemoglobin A (1HHO)

Electron Transport: Rubredoxin (2RXN)

Structural Support: Zinc fingers in transcription factors (1PYI)

Metalloenzymes: Superoxide dismutase (2SOD)

For each of the above proteins, we will use structural information and visualization to glean important information about the metal coordination environment and its effect on the local protein structure.

- 1). Access the Protein Data Bank (PDB) at <http://www.rcsb.org>.
- 2). Type in hemoglobin into the search window. You can see that there are many different results returned (over 36 pages!), all of which contain hemoglobin. It is important to pay attention to detail and select the correct structure set.
- 3). Enter the keycode 1HHO. This will take you directly the dataset for a high resolution structure of oxyhemoglobin. The left side of the page contains links from which you can download files for future reference. Additionally, we could download the sequence of the metalloprotein (FASTA link).
- 4). Click on the *Display Molecule* link on the left drop down menu. We have several choices of how to view the molecule. Start by clicking on the top link: *Image Gallery*.
- 5). The *Image Gallery* gives us some static pictures of the hemoglobin as well as a primitive interactive applet. Manipulate the applet until you can easily see the heme unit and the coordinated dioxygen molecule.
- 6). Select the *KiNG* viewer link from the left menu. This applet gives us more control over the viewing of the structure.
 - a. Practice manipulating the structure with your mouse. You can see the two different subunits are colored differently.

- b. Let's focus on the metal center. Drop the ribbon out of the picture by deselecting the "ribbon" link from the right side. (Alternatively, we could have removed the α helices by clicking on "helix".) Now you should see only the prosthetic heme units, along with metal ions and dioxygen.
 - c. Zoom in on one of the metal centers. Examine the geometry around the metal center.
 - d. Reintroduce the ribbons and slowly zoom out. Note the local environment around the prosthetic group.
- 7). Next, launch the *Jmol* program. This program is a no-frills visualization tool similar to the one available under the *View Image* link.
- 8). Launch the *MBT Protein Workshop* applet. This applet provides several user friendly tools for examining structure and domain types.
 - a. Select the *Shortcuts* tab at the top of the page. The default setting is *Chain Color Ramp*. Switch this to *Conformation Type* and click *Enact*. Now local conformations are color coded for easy identification.
 - b. Switch the color coding to *Hydrophobicity*. Locate the most hydrophobic residues. Are they on the surface of the protein or internalized?
 - c. Finally, click on the *By Compound* link. This will show the location and identity of different amino acid residues in the protein. If desired, you can open the sequence information and compare it to the display.
- 9). Finally, select the *WebMol* visualization program from the left link. This program offers features not available in other viewers.
 - a. First, select the *Rock* button. This will put the protein in motion.
 - b. Try clicking on the *HOH* button. This will display the location of water molecules that were present in the solved structure.
 - c. Click on the *Labels* button. This will show a messy picture of all the labels on the protein. Although not useful at this resolution, it can be very useful when looking at a smaller section of the protein.
 - d. Select the *Backb* link from the upper right drop down box. This will switch the view to a skeleton of the structural backbone.
 - e. Using the *Focus* button, select the prosthetic group. Select 10 Å as the range for the focus. You should see the prosthetic group and the immediate protein environment around it. This may take a few tries if you were not correctly centered on the prosthetic group. Once you have a centered image, click on *Labels* again. Can you identify the amino acid residue bound to the metal center?
- 10). Use the above visualization tools to view the other three metalloproteins as well.

Laboratory Report:

1). For each of the four metalloproteins, use the visualization tools to answer the following questions:

- Metal(s) present? How many metal atoms per protein?
- What domain types are present in the metalloprotein?
- How many subunits does the metalloprotein contain?
- Are prosthetic groups present? If so, how many?
- If prosthetic groups are present, identify the amino acid residues that interact with the metal and/or prosthetic group itself.
- Describe the hydrophilic/hydrophobic nature of each metalloprotein's surface.

2). Select a metalloprotein from lecture and search the PDB for it. Once you have identified the appropriate structural file, use the different viewers to answer the questions listed above.

3). Develop a short users guide for the viewer programs. Describe what you perceive to be the strengths and weaknesses of each of these applets. Focus on which applet you would recommend for different viewing tasks.