Department of Chemistry,
The University of Western Ontario

Chemistry 9621A: Methods in Bioinorganic Chemistry

September-December, Fall 2021

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Enrollment: Max 12

Description: This 1/2 course introduces the inorganic chemistry of biological molecules by describing essential, toxic and metallopharmaceuticals and specific examples of metalloproteins that depend on metal-based chemistry for function. Studies of metalloproteins involve many techniques and the course includes the background to several of these techniques and methods emphasizing the specific sensitivity to metals coordinated to biological molecules. **Topics include**: Introduction to metal-based biological chemistry (bioinorganic chemistry), with examples of metalloprotein structure and metal-related properties. Optical spectroscopic methods: absorption, CD, MCD, emission (including lifetimes/quenching). Mass spectrometry – especially the electrospray ionization soft methods for proteins and complete metal complexes. Metal concentration analysis via atomic absorption and emission. A brief section on chromatographic methods using size exclusion columns. Synchrotron radiation based methods – especially EXAFS and XANES. Theoretical methods: Molecular mechanics (MM); molecular dynamics (MD); simple MO; more complete results using DFT and TDDFT methods.

Lecture dates Tue/Thur 4:00 – 5:30 pm. Starting Sept 21st - time can be adjusted to suit TA assignments.

The Course – TOPIC overview

Part 1) Introduction to Bioinorganic Chemistry - typical molecules

Essential, toxic and metallopharmaceuticals. Common biological metal binding sites – identifying the donor atoms in amino acids. Examples of three metals to illustrate the issues in determining the mechanistic details of biological reactivity (Zn, Co, Fe). Zn in alcohol dehydrogenase, Co in cobalamin (vit B12) as examples of binding sites for Zn(II) and Co(II). And the role of the heme porphyrin in heme proteins.

Part 2) Physical and theoretical methods used to study metallobiological molecules

Methods used to determine structure and function – in other words – the mechanistic details of the biological reactivity. What are the goals of the method/techniques used in this course? : Metal:Ligand stoichiometries, so numbers of metals bound per protein molecule; then equilibrium constants. Protein structures as a function of metal ion binding or denaturant. Structural and Electronic properties – reactivity control – use of molecular dynamics to probe large molecule structures and MO-methods to probe the electronic structure responsible for redox and spectroscopic properties.

Lecture TOPICS in order Lecture notes will be provided for each unit.

1. Introduction – overview of course lectures and exercises
2. Bioinorganic chemistry – the vital role of metals
3. Atomic absorption and atomic emission; ICP-MS - for metals (applies to Assignment 1, which can be started after this unit)
4. Metals ions control life in many ways from simple ionic strength to complex catalysis
5. Metals of interest: essential, toxic, and pharmaceutically important metals
6. Bioinorganic Chemistry – introduction to key Inorganic chemistry
7. Refresher – Hard-Soft Acid – Base concepts control likely coordination properties of metals to biological ligands
8. Equilibrium constants (K_F) control the distribution of metal ions amongst thousands of possible hosts
9. Bioinorganic Chemistry – introduction to key biochemistry
10. Amino acids – common donor atoms for metal ions
11. Proteins – structural details
12. Bioinorganic Chemistry – metalloprotein cases
13. The porphyrins, especially heme and chlorophyll. Iron in heme proteins
14. Optical spectroscopy: absorption, CD, MCD, emission (including lifetimes/quenching) – (applies to Assignment #2, which can be started after this unit))
15. Comparisons between cytochrome c (straight redox), horseradish peroxidase (powerful oxidizing agent), myoglobin (O_2 storage in muscles) and hemoglobin (O_2 transport from lungs to muscles).
16. How do these very different metalloproteins exploit the properties of iron?
17. Mass spectrometry – especially electrospray ionization – for proteins and metal binding (applies to Assignment #3, which can be started after this unit)
18. Cobalt in cobalamin, commonly known as the water soluble vitamin B12
19. Zinc in thousands of proteins, examples, alcohol dehydrogenase, and zinc fingers – a wonderful application of a potentially inert d^{10} metal ion!
20. Theoretical methods (part 1): Molecular mechanics MM; dynamics MD
21. Theoretical methods (part 2): Simple MO; DFT for ground state geometries and TDDFT for calculating absorption spectra – (applies to Assignment #4)
22. Chromatography: Size exclusion columns - for proteins and large molecules
23. Synchrotron methods – especially XANES/EXAFS – for bond lengths; donor atom identities; coordination numbers (less precisely) (the extent of this unit depends on the available lecture time)

Evaluation/Assessment There are no quizzes, tests or exams. The assessment is based on 4 presentations and written lab reports describing 4 hands-on, in-person experiments carried out by each participant at the own time and pace. Deadlines to be confirmed depending on the enrollment, but provisionally, in class time in weeks 5, 7, 10 and 13 (last week of the class).

This form of assessment is based on “putting theory into practice”, by completing four in-person, hands-on experiments using the different instrumental methods described in the lectures and reporting the results in presentations. Full background, training and experimental procedures will be provided in the course for each experiment. (Participants can bring their own samples for analysis or molecules for use in the MM/MD or DFT/TDDFT experiments or use samples and molecular structures I provide.)

Training will be provided for each instrument and method, and your use of the instrument can be in times convenient to you (booking sheets will be available). The estimated “lab” time should be less than 3 hours followed by a report. The samples to be used will be provided or you can use your own from your research (following my approval)..

Presentations describing the experimental results and putting the technique into context will take place within the lecture times on either Tuesday or Thursday approximately every 2 weeks starting on October 15th following the

background lecture and training.

Evaluation/Assessment: Details of 4 Hand's On Exercises plus associated Short Presentations (see note below for mark distribution)

EXPT 1 – 15% "AAS –METAL ANALYSIS" Use the atomic absorption instruments (AAS) to determine the concentrations of two metals in a solution.

EXPT 2a – 25% : EMISSION methods. Use emission spectroscopy to identify an aromatic amino acid in a protein and then determine its approximate distance inside the protein using fluorescence quenching caused by a paramagnetic metal ion (Stern Volmer technique)

or

EXPT 2b – 25%: Circular dichroism expt: Unfold the highly folded heme protein myoglobin using acid watching for the release of the heme and the complete loss of the folded structure. (Could also compare this traditional use of circular dichroism spectroscopy with ESI-MS data to quantify the presence of alpha helical structure – there are 8 helical regions in myoglobin.)

EXPT 3 – 30%: Measure the ESI-MS spectrum showing and explaining the native and denatured properties of a protein supplied by MJS (full instructions provided at class). **Those taking the course with extensive experience of ESI-MS will be assigned an alternate exercise requiring measurement of the CD, emission, excitation and excited state lifetime of a protein under native and denaturing conditions – protein to be provided by MJS (full instructions provided at class).

EXPT 4A – 30%: Use molecular modelling (MD) to study how short peptides fold by including metal ions bound in typical coordination geometries. Use molecular dynamics (MM-MD) to watch the denaturation of a protein.

OR

EXPT 4B – 30%: Use DFT and TDDFT methods to calculate a minimized geometry showing the electron distribution in the highest occupied and lowest unoccupied MOs, then predict the UV-visible absorption spectrum.

Presentation (less than 10; 15; 20; 20 minutes) and **Lab report** (less than 4 single sided 1.5 lines pages) structure (the oral Presentation is based on and may expand the written Lab report): Part 1: description of the instrument and method; then Part 2: your results; then in Part 3: the Discussion introduce a published paper that uses similar methods in a metalloprotein or other bioinorganic setting (very wide range of papers acceptable.)

The intention is the develop skills that allow description of complicated experimental data to a general audience (here your fellow participants), based on the concept that hearing different words about something you know about cements your knowledge. Grading is based equally on the quality of your results and the quality of your descriptive and explanatory text.

As mentioned above: you can bring you own samples and molecules to Expts 2, 3 and 4 as long as they adhere to the conceptual focus of the Expt: in (2) sensitivity of excited states to paramagnetic interactions, in (3) protein unfolding, in (4) molecular structure and electronic control of optical properties.

Software access if required will be made available to all participants (using software in your own lab is perfectly ok); ESI-MS machine time will be available to match your availability following a training session (using your lab's emission, CD, and/or ESI-MS instruments is quite acceptable).

The Course: Detailed TOPICS (not the running order – see below)

1) Bioinorganic Chemistry – the vital nature of metal ions
- Metals ions control life in many ways from simple ionic strength to complex catalysis
- Metals of interest: essential, toxic, and pharmaceutically important metals

2) Bioinorganic Chemistry – introduction to key Inorganic chemistry
- Refresher – Hard-Soft Acid – Base concepts control likely coordination properties of metals to biological ligands
- Equilibrium constants (KF) control the distribution of metal ions amongst thousands of possible hosts

3) Bioinorganic Chemistry – introduction to key biochemistry
- Amino acids – common donor atoms for metal ions
- The porphyrins, especially heme and chlorophyll
- Proteins – structural details

4) Bioinorganic Chemistry – metalloprotein cases
- Cobalt in cobalamin, commonly known as the water soluble vitamin B12
- Zinc in thousands of proteins, examples, alcohol dehydrogenase, and zinc fingers – a wonderful application of strictly inert metal ion!
- Iron in heme proteins
- Comparisons between cytochrome c (straight redox), horseradish peroxidase (powerful oxidizing agent), myoglobin (O_2 storage in muscles) and hemoglobin (O_2 transport from lungs to muscles).
- How do these very different metalloproteins exploit the properties of iron?

5) Methods
- The necessity of a range of methods to characterize and understand the reaction properties of metalloproteins
- What are the goals: Metal stoichiometries-concentrations; ligands-donor atoms; complexes – binding sites; metals-electronic properties – reactivity control

6) Physical and theoretical methods used to study metallobiological molecules
- Atomic absorption and emission - for metal concentrations
- Optical spectroscopy: absorption, CD, MCD, emission (including lifetimes/quenching)
- Mass spectrometry – especially electrospray ionization methods (ESI-MS)
- Theoretical methods: Molecular mechanics; dynamics; simple MO; DFT
- Chromatography: GC and HPLC; SEC Resonance methods – Raman and resonance Raman
- Synchrotron methods – especially EXAFS and XANES

7) Assessment

There are no quizzes, tests or exams

The assessment is based on 4 presentations and written lab report (to be confirmed depending on the enrollment, but provisionally, in class time in weeks 5, 7, 10 and 13 (last day of the class).

Training will be provided for each instrument and method, and your use of the instrument can be in times convenient to you (booking sheets will be available). The estimated "lab" time should be less than 3 hours. The samples to be used will be provided.

(1) "AAS –METAL ANALYSIS" Use the atomic absorption instruments (AAS) to determine the concentrations of two metals in a solution.
(2) "EMISSION – Use emission spectroscopy to identify an aromatic amino acid in a protein and then determine its approximate distance inside the protein using fluorescence quenching caused by a paramagnetic metal ion (Stern Volmer technique)
(3) Unfold the highly folded heme protein myoglobin using acid watching for the release of the heme and the complete loss of the folded structure. (Could also compare these ESI-MS data with the traditional use of circular dichroism spectroscopy to quantify the presence of alpha helical structure – there are 8 helical regions in myoglobin.)
(4) Use molecular modelling (MD) to study how short peptides fold by including metal ions bound in typical coordination geometries. Use molecular dynamics (MM-MD) to watch the denaturation of a protein.

RESOURCES

Recommended text Books (available in the Science Library and also available from mjs)

Bioinorganic chemistry : a short course by Roat-Malone. QU130.R628b

Bioinorganic chemistry : inorganic elements in the chemistry of life : an introduction and guide by Kaim and Schwederski.

The biological chemistry of the elements : the inorganic chemistry of life by da Silva and Williams. QU4.S586b 2001 A rather different book in which the evolution of biological materials that incorporate metal ions is discussed in details. A very good read.

Biological Inorganic Chemistry – Structure and Reactivity by Bertini, Gray, Stiefel, and Valentine (2007) TAYSTK QU ??? 2007. An exceptional book if you are planning on 4th year research or graduate work on topics that involve metals in biology. Has no chapters on toxic metals; very brief on metals in medicine. .

Concepts and Models in Bioinorganic Chemistry by Kraatz and Metzler-Nolte. TAYSTK QU ??? 2006. Very interesting description of the key metal-ligand regions by discussing small molecule models of biological molecules.

QD96.E44L49 1984: Inorganic electronic spectroscopy / A.B.P. Lever
QD96.V53H37 1978: Symmetry and spectroscopy / D.C. Harris, M.D. Bertolucci
QD471.F57 1961: Introduction to ligand fields / B.N. Figgis
QP531.P47 2000: Physical methods in bioinorganic chemistry / ed. L. Que, Jr.
QD462.C653 2000: Computational molecular spectroscopy / ed. P. Jensen and P. Bunker
QD95.I486 1999: Inorganic electronic structure and spectroscopy / eds. Solomon, Lever
QP531.L55 1994: Principles of bioinorganic chemistry / eds. Lippard, Berg
QP531.B543 1994: Bioinorganic chemistry / eds. Bertini, Gray, Lippard, Valentine
Updated 2013 Sept 9 Rev 13-3

Molecular Modelling:

CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations, J. Comp. Chem. 4, 187-217 (1983), by B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus.

CHARMM: The Energy Function and Its Parameterization with an Overview of the Program, in The Encyclopedia of Computational Chemistry, 1, 271-277, P. v. R. Schleyer et al., editors (John Wiley & Sons: Chichester, 1998), by A. D. MacKerell, Jr., B. Brooks, C. L. Brooks, III, L. Nilsson, B. Roux, Y. Won, and M. Karplus.

CHARMM .doc Home NIH CHARMM .doc Home.

CHARMM related documents - Links to CHARMM official documents and example files ...etc.

CHARMm Principles - A nice introductory document about CHARMM and molecular modeling included in MSI's (now Accelrys) Insight II package. This link points to a copy on Taiwan ASCC website.

The CHARMM Water-Box Tutorial - A hands-on example of using CHARMM to run a box of water. Not really useful, but certainly helps when one wants to get started.

The CHARMM Butane Tutorial - Continues the previous water-box tutorial. Includes introduction to analyzing data using gnuplot and vmd.

MAMD Workshop - Contains lectures and tutorials given in the Workshop on Methods and Applications of Molecular Dynamics to Biopolymers (June 4 -7, 2000).

Preparation of Protein system from initial pdb file - Michael Crowley's tutorial on how to modeling a protein using CHARMM start from a pdb file.

CDAC CHARMM Tutorial - CHARMM tutorial on the Center for Development of Advanced Computing website.

Charmm Notes - Georg Schreckenbach's notes on running CHARMM.

CHARMM force fields - Alex MacKerell's research site about CHARMM force fields.

CHARMM.pm - You guess what this is. :)

WIDTH - A simple CHARMM histogram utility.

ptraj/rdparm - Software that can process CHARMM/AMBER trajectory files.

Background Information – no need to use any of these – this is just to help you set up your answers.

PDB File

The Protein Data Bank (PDB) is now THE protein structure archive of the world. The archives provide atomic coordinates and other useful information (bibliographic citations, primary and secondary structure information, quality of the crystal... etc.) in pdb file format. One can find a complete description of the PDB format here:

PDB File Format and Standards

Most of today's chemical structure viewers support pdb file and can display the beautiful 3-D structure of macromolecules without any problem. Nevertheless, the most popular cross-platform pdf file viewer might be Rasmol. Rasmol is a freeware, and can be downloaded from the RasMol Homepage . For people who likes to try out other options, this site on Free Molecular Visualization Software is quite comprehensive.

•Swiss-PdbViewer program by Nicolas Guex and Manuel C. Pietsch. This program runs on Macintosh or Windows NT/95 (but not Windows 3.1x). You can overlay and align multiple structures, mutate amino acids (the best rotamer of the new AA is found automatically), and dynamically find/display hydrogen bonds. Upgraded to version 2.0 March, 1996 (first Windows version; Mac version now supports Quickdraw3D).

http://www.umass.edu/microbio/rasmol/molmol.gif

•MolMol (Win95, unix) makes publication quality images including surfaces, and has a graphical user interface with menus, dialog boxes and on-line help, including a hypertext manual and tutorial. Especially good support for multi-model NMR files. Supports limited modeling (addition or removal of atoms/bonds, and rotation around dihedral angles).

•Cn3D (Read as See in 3D) is a superb new free viewer from the National Center for Biotechnology Information. It uses "cleaned-up" PDB files converted to ASN.1 format available thru WWW Entrez, also available directly within Cn3D which will itself query the Entrez database. Considerable effort has been applied to the ASN.1 data files to resolve ambiguities present in the PDB format and remove errors present in some PDB files. See the on-line article by Hogue et al. for an explanation. Cn3D is more intelligent than RasMol -- for example, it provides a more informative first image of a structure.

•The Molecule to Image Converter Engine by Jozsef Ferincz is an amazing web form which allows you to submit a PDB file URL, and get back a RasMol image!

•MolView and MolView Lite are described by their author, Thomas J. Smith, as follows. Freeware programs written for the Mac. The user cannot only display proteins in all of the various ways but can also analyze them (e.g. distances, Ramachandran plots, Edmunson wheels, hydrophathy plots, 3D structure alignments). Ribbon drawings can be drawn in many different ways - all controlled by graphical buttons. Subsets of structures can be written out to 'MOL files' to be read in at a later time. While there is more flexibility in display options than other programs, the interface is entirely graphically based and not script driven. In contrast to most other programs, the output images are mostly kept as object-oriented PICT files for very high quality prints. Finally, the user can also output 3 different kinds of QuickTime movies, line DXF files, and Apple's new 3DMF files for interactively rendered ball&stick, ribbon, space filling, line, and 'tube' models.

Conversion/manipulation of atomic coordinate data files

•Babel by Pat Walters and Matt Stahl (U AZ, Tuscon) interconverts many atomic coordinate data file formats (e.g. Alchemy to PDB or vice versa). It runs on Windows, Macs, and unix systems. It can also assign hybridization, bond order, and connectivity when these elements are not present in the input file. If you just need a few quick conversions, Jozsef Ferincz has provided an amazing web form you can fill out -- the converted file will appear in your browser and can be saved to disk! It is the Molecular Modeling Interconverter Engine.

•Hingefind is an X-PLOR script by Willy Wriggers which identifies domain movements in proteins. It generates output files which can be visualized with standard software.

•Computational Center for Macromolecular Structures, CCMS, at the San Diego Supercomputer Center. Here you can get free programs including SHAPE (analysis of molecular surfaces), FLEX (molecular animations), PDBtool (browser), XTALVIEW (X-ray crystal structure solver). These are mostly for unix/workstations/xwindows systems.

Protein Explorer, a RasMol-derivative, is the easiest-to-use and most powerful software for looking at macromolecular structure and its relation to function.

What is the PDB?

The Protein Data Bank (PDB) is the single international repository for public data on the 3-dimensional structures of biological macromolecules. The contents are primarily experimental data derived from X-ray crystallography and NMR experiments. The primary goals of this resource are:

- To enable you to locate structures of interest;
- To perform simple analyses on one or more structures;
- To act as a portal to additional information available on the Internet;
- To enable you to download information on a structure, notably the Cartesian atomic coordinates, for further analysis.

Course attendance and missed/late assignments

If you are unable to meet a course requirement due to illness or other serious circumstances, you must provide valid medical or other supporting documentation to your instructor immediately. It is the student's responsibility to make alternative arrangements with their instructor once the accommodation has been approved and the instructor has been informed.

Notes on Academic Honesty

Scholastic offences are taken seriously and students are directed to read the appropriate policy, specifically, the definition of what constitutes a Scholastic Offence, at the following Web site:

www.uwo.ca/univsec/pdf/academic_policies/appeals/scholastic_discipline_grad.pdf

All required papers may be subject to submission for textual similarity review to the commercial plagiarism detection software under license to the University for detection of plagiarism. All papers submitted for such checking will be included as source documents in the reference database for the purpose of detecting plagiarism of papers subsequently submitted to the system. Use of the service is subject to the licensing agreement, currently between The University of Western Ontario and Turnitin.com (http://www.turnitin.com).

Health and Wellness

As part of a successful graduate student experience at Western, we encourage students to make their health and wellness a priority. Western provides several on campus health-related services to help you achieve optimum health and engage in healthy living while pursuing your graduate degree. For example, to support physical activity, all students, as part of their registration, receive membership in Western's Campus Recreation Centre.

Numerous cultural events are offered throughout the year. For example, please check out the Faculty of Music web page http://www.music.uwo.ca/, and our own McIntosh Gallery http://www.mcintoshgallery.ca/. Information regarding health- and wellness-related services available to students may be found at http://www.health.uwo.ca/. Students seeking help regarding mental health concerns are advised to speak to someone they feel comfortable confiding in, such as their faculty supervisor, their program director (graduate chair), or other relevant administrators in their unit. Campus mental health resources may be found at http://www.health.uwo.ca/mental_health/resources.html