“Molecular Analysis of Natural Systems” CHM 1404

This course will be offered at UTSC, and will be 4hrs biweekly.

Date	Weeks	Topic	Notes	Room
Jan 12th	1-2	Lectures introducing Basic NMR – NMR tutorial	Set Assignment 1;	Quercus Online 10am-2pm
Jan 26th	3-4	Lectures on Structure and Aggregation in Environmental Research	Present Ass 1;	Quercus Online 10am-2pm
Feb 9th	5-6	Practical 1 – Introduction to Practical NMR		Quercus Online 10am-2pm
Feb 23rd	7-8	Lectures on Molecular Interactions by NMR – Contaminant Transport Fate, Binding and Sequestration.	Set Assignment 2	Quercus Online 10am-2pm
Mar 9th	9-10	NMR and Statistics Sensitivity Enhancement, Novel Applications and the Future on NMR Spectroscopy.	Present Ass 2; Set Take Home Exam	Quercus Online 10am-2pm
Mar 23rd	11-12	Fun Practical 2 - Multiphase NMR (Bring your own natural samples) Advanced Calibration – quantitative data.	Hand in take home exam	Quercus Online 10am-2pm

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Prerequisites: permission of the instructor

Goals :

1) To encourage students to consider how they can utilize traditional and emerging analytical techniques synergistically and design new analytical approaches to address the role of complex systems in the environment. Emphasis will be place on NMR spectroscopy and hyphenated NMR spectroscopy.

2) To introduce the *environmental applications* of NMR spectroscopy, hyphenated NMR, imaging and related computation techniques (prediction, simulation, elucidation), such that students have a basic grasp of the subjects, and can relate the potential of the approaches to their own research. The emphasis will be on environmental and biological applications. Theory will be explained such that students can fully grasp cutting edge applications. However, the course will place emphasis on accessibility such that students without a strong NMR background are comfortable in the class. The class will include a full recap of NMR practice and theory to give everyone an equal footing.

Student Evaluation:

Research assignments/Lab Reports	40%
Presentations	20%
Take Home Exam	40%

Students will be required to submit and present a “research” assignment which is based on the research topic for that section. Students will be encouraged to relate research assignments on their own research. Import analytical questions/problems that arise from the reports will be discussed at the beginning of the class.

In addition, to lectures every week a range of other methods will be used to get you involved in your own learning these will include :

Hands on tutorials: An NMR tutorial will be held at the end of the first section. This tutorial will give you experience with complex NMR datasets and structural assignment.

Practical Sessions: The first practical is very structured and well cover all aspects of solution state NMR of mixtures, including identification, quantification, spectral simulation, interpretation. The goal is to introduce you to different forms of NMR, put theory into practice, and show you NMR of mixture is a powerful tool. The first practical we will use conventional solution state NMR.

In the last practical we will attempt real samples using “Comprehensive Multiphase NMR”. This is novel NMR technology that permits, solution-state, gel-state and solid-state NMR. You will be given the opportunity to bring in any sample you like. Have a think it needs to be ~3.2mm or less in width and less than 1cm in length and ideally contain more than one phase. Examples could be seeds, food, materials, swellable materials, biological process (no biohazards) etc. We can also try small living invertebrates to demonstrate the potential of in-vivo MAS NMR. Feel free to bring in a sample and we will run as many as

possible and discuss the types of information we can obtain on whole unaltered samples. In this practical will be asking more research questions such as what information can NMR provide on unknowns, as-well as learn a range of advanced practical methods and spectral editing approaches, solution, gel and solid-state NMR.

Great NMR Challenge: A fun NMR quiz will be held at the end of week 8. We will study NMR of food, beverages, and common products from around the world. We will combine general knowledge, NMR interpretation and simulation to try to guess what the products are. This will not be graded but the person with the highest mark with get a Tim Horton's gift certification.

Challenge Questions: At the end of week 10. We will tackle the challenge questions. These are big picture questions that require in-depth thinking and require you to use and combine all the knowledge you have gained during the course. You will split into small groups to design and develop the bet way to answer the question. You will present your approach and argue why it is better than the other competing teams. This is a fun and challenging way to get you to work together, think outside the box and develop ideas that ultimately could solve very large questions in science.

Logistics:
To accommodate students from throughout the tri-campus system, the class will meet every 2 weeks for 4 hours. With the exception of the lab session which will last *all day*. This course will be offered at UTSC.

Reading : Key articles in the literature will be used as reading.

Course Outline /Syllabus :

Week 1-2: **Course orientation**

- Introduction to complex mixtures and heterogeneous systems
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Week 1-2 : *Part lecture/part hands on tutorial. Complete recap and NMR theory and practice. Including a 2 hrs of lectures and 2hrs of hands on tutorials.*

In addition to conventional NMR this week will also introduce the main analytical problems associated with working with complex mixtures and heterogeneous systems. We will consider a range of systems pertinent to everyone's research, including, air particulates, surface films, dissolved organic matter, sediments, and soils. Emphasis will be placed on the importance of primary structures (molecular), and "secondary structure" (formation of colloids, organic/inorganic associations, physical conformation) in order to understand environmental reactivity both in context of contaminant interactions and the global cycling (carbon, nitrogen cycles).

Assignment will focus on traditional analytical approaches that have been employed to date.

Week 3-4: **Introduction to Environmental NMR: Structure**

Week 2-3 : Part lecture/part presentation of assignment. This week will introduce basic NMR in the context of environmental chemistry. This week will address the basic questions :

What structural information can NMR provide ?

Chemical Structure
Molecular Weight and Aggregate Size
Surface Chemistry, and Macroscopic Order
Folding, Conformation

What sort of samples can be studied ?

Solution NMR
HR-MAS NMR semi solids
Comprehensive Multi-phase NMR
Solids NMR
Imaging

- Prediction and Simulation

Assigning structural fragments and compounds within mixtures
Identification of unknowns without libraries
Quantification in Mixtures
Databasing and Pattern Matching
Computer Assisted Structural Elucidation (CASE)

Assignment will focus on Molecular Interactions.

Weeks 5-6: Laboratory Practical 1

Practical session to demonstrate basics of mixture analysis using solution-state NMR. Understand a wide range of practical aspects including :

- Sample Preparation
- Solution NMR
- Calibration
- Quantitative Conditions
- T1 measurement
- 1D and 2D collection and interpretation.
- Dispersion, Overlap
- Limits of Detection and Quantification
- Electronic Referencing for Quantification (ERETIC)
- Data processing and Visualization
- Prediction and Simulation
- Full spectral assignment and quantification in mixtures.

Assignment will be a lab report including appropriate calculations and methods.

Weeks 7-8: Molecular Interactions/Hyphenated NMR

Week 5-6 : Part lecture (2hrs)
Interpretation Quiz to recognize diverse samples (includes a prize)
Challenge questions – work as a group to tackle some of the largest questions in science using knowledge learnt in class.

Topics covered include :

- Binding Models
- Covalent Non-covalent Interactions
- Chemical Shift Perturbations
- DOSY, diffusion dynamics
- Relaxation Studies
- Overhausser Effects, NOESY, ROESY
- Saturation Transfer – Epitope Mapping
- Reverse Saturation Transfer – Binding Sites
- Surface Binding - WaterLOGSY
- Cross Polarization
- Double Cross Polarization, REDOR

- Introduction to hyphenated approaches (LC-NMR, LC-SPE-NMR/MS)
- NMR as a chromatographic detector
- Isolation of contaminant complexes from natural mixtures
- Hyphenated NMR as a precursor for the structure elucidation of novel compounds

- Emerging Hyphenated Methods (CE-NMR, GC-SPE-NMR)

Assignment will focus on the present applications of Hyphenated NMR and measurement of molecular interactions within Environmental/Biological Chemistry and the potential for future applications.

Weeks 9-10: Related Computational Methods/Sensitivity Enhancement/Metabonomics/Cutting Edge Applications/High through put screening

High throughput/NMR screening

- Introduction to multivariate statistics
- Finding correlations between diverse datasets
- Identify key compounds within complex mixtures
-Relating Molecular and Global Scale Phenomena
- Targeted and Non-targeted Analysis, Stats, Metabolomics
- PCA, PLS, loading plots, T-test filtered 1D and 2D NMR

Sensitivity Enhancement

- DNP,
- Cryo Probes,
- Hyperpolarized Gases,
- Micro-coils,
- Parahydrogen
- Non uniform sampling
- Multiple Receivers

Applications in :

Food Screening, Juice and Wine Screening and Fraud

Medicine,

- Olympic Athlete monitoring,
- cancer surgery,
- aging metabolome,
- disease and new born screening
- heart monitoring, and lipidomics.
- Environmental

In-vivo NMR and environmental stress.

- Toxic Mode of action,
- Nutrition
- Contaminant Exposure

Assignment will focus on the potential for future applications.

Weeks 11-12: Laboratory Practical 2

Practical session to demonstrate in-situ NMR on real unaltered samples. Hands on experience including organisms *in-vivo*.

- Sample Preparation
- Flow NMR
- In-vivo NMR
- Isotopically Enriched Samples
- Advanced water suppression
- Water Suppression in 2D
- Metabolic Fingerprinting
- Data processing and Visualization
- Prediction and Simulation
- Demonstration of Elucidation and Statistics

Assignment will be a lab report including appropriate calculations and methods.

Weeks 12-13: Context, Overview and the Future

- Work Flow (using methods appropriately and efficiently)
- Combining traditional with emerging analytical approaches
- Present Limitations
- Thinking outside the box (Multidisciplinary Approaches)
- Future Directions

Take home exam will cover the most important principles described during the course.

Compulsory Reading to be read by the class indicated.

In addition to reports and assignments there will be some compulsory reading. 1 journal article per session : You must have read and understood this we may discuss these in class. *Please note the articles should be read before the class stated. For example we may discuss the Simpson M. J. paper in Weeks 3-4 (the second time be meet).* The articles are as follows

Weeks 1-2

Cardoza, L. A., et al. (2004). "Applications of NMR spectroscopy in environmental science." *Progress in Nuclear Magnetic Resonance Spectroscopy* 45(3-4): 209-238.

Week 3-4

Simpson, A.J., McNally, D, Simpson, M. J., "NMR Spectroscopy in Environmental Science : From molecular-level interactions to global processes". *Progress in Nuclear Magnetic Resonance Spectroscopy*, 2011, 58, 97-175.

Weeks 5-6

Simpson, A. J. (2002). "Determining the molecular weight, aggregation, structures and interactions of natural organic matter using diffusion ordered spectroscopy." Magnetic Resonance in Chemistry 40: S72-S82.

Weeks 7-8

Jun Kikuchi, Kengo Ito, Yasuhiro Date (2018). "Environmental metabolomics with data science for investigating ecosystem homeostasis." *Progress in Nuclear Magnetic Resonance Spectroscopy* 104 (2018) 56–88

Week 9-10

Simpson, A. J., et al. (2004). "The application of LC-NMR and LC-SPE-NMR to compositional studies of natural organic matter." Analyst 129(12): 1216-1222.

Weeks 11-12

John C. Lindon, Elaine Holmes, Jeremy K. Nicholson Progress in Nuclear Magnetic Resonance Spectroscopy "Toxicological applications of magnetic resonance"45 (2004) 109–143

General Class Outline

Formal Lecture components ~ 2hrs per week.

Class Participants will present their research from the assignments (1hr) or Interactive Learning Exercise (1hr)

Class discussion (~1 hr)

Introduction to a new topic

Discussion

Set a research assignment for all of you

Practical Session

The first practical is very structured and well cover all aspects of solution state NMR of mixtures, including identification, quantification, spectral simulation, interpretation. The goal is to introduce you to different forms of NMR, put theory into practice, and show you NMR of mixture is a powerful tool.

The first practical we will use conventional solution state NMR.

In the last practical we will attempt real samples using “flow NMR”. This is novel NMR technology that permits, living organisms to be kept alive inside the spectrometer. In this practical will be asking more research questions such as what information can NMR provide in-vivo, experiments to simplify data, advanced water suppression, and other more advanced concepts

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Marks for this course will be allocated as follows :

Assignments x 2 = 20% each = 40% Total
Presentation x 2 = 10% each =20% Total
Take Home final = 40% Total

You will be given the option to select your own assignment is there something related to NMR you would like to learn about ? If so you can choose this to be your assignment. If not you can select from a list.

Assignments

Each assignment must be limited to 2 pages total including references and Figures. It should be easy to read and cite the key references. Figures should be used only to highlight an important concept. But figures used correctly "are worth a 1000 words" If there is not Figure in the literature maybe you can draw one to help get the message across.

Each assignment should be split into the following sections here is an example :

Title : "Can Br NMR be used to study Flame Retardants in the Environment"

Background (20%)

Why are flame retardants important environmentally ?

Major Results and Examples from the Literature (40%)

Has Br NMR ever been applied to anything environmental.
Discuss why you think it has or has not.

If Br NMR has never been applied to environmental has it ever been used in any field . Does it show potential for environmental applications.

Impact on your research/interests (or the field in general, as applicable) (20%)

How do you think it could impact you research. How could it help. If you think it could not impact you research you must justify why not.

If it is not your area, how have the applications impacted the field.

Feasibility and Future (20%)

Think about it carefully is this an area that has huge application ? Or is it really not feasible, perhaps due to sensitivity. Would special or novel hardware be needed ? Give your perspective. Marks are given for valid argument backed up scientifically.

Presentation **(10mins presentation, 10mins discussion)**

You will have to make copies of your 2 page report for everyone to read. This is your responsibility. The presentation will be informal you can take us through your report and explain what you found.

You may also prepare up to 10 powerpoint slides if this helps with your presentation. In addition, you can also present a few power-point slides. Slides are not compulsory, but recommended. Do not duplicate things in your report on slides. Use slides to help you communicate your point to the group where applicable. The goal is to communicate in an ***understandable*** fashion to a non-expert your topic.

After each talk instead of conventional questions, we will have an informal discussion, to talk about potential applications, understand the topic better, future potential etc.

Marks for Presentation :

2% Quality of extra material required for presentation, slides, diagrams etc..
2% Readiness to discuss present your data.
2% Quality and Clarity through which you present your findings.
2% Depth of knowledge/Ability to answer questions.
2% Ability to stay within time frame.