BB 651—Winter 2016

Macromolecular NMR Spectroscopy

Prerequisite: BB481/581 and BB490/590 or consent of instructor

Professor: Dr. Elisar Barbar, 2133 ALS (737-4143; barbare@oregonstate.edu)

Course Hours: T,Th 8:30 – 9:50 a.m. ALS 2040

Course Objectives: NMR spectroscopy is the only method for high-resolution structural determination of proteins that do not form diffracting crystals, and for identifying transient structures in intrinsically disordered proteins. NMR is also the only method for identification of protein interfaces in dynamic complexes and of conformational changes that accompany protein complex formation. The goal of this class is to cover both theory and practical applications of NMR for students who plan on using NMR in their research. NMR techniques to be discussed include *in vitro*, and *in silico* methods.

Course Purpose: To provide an in depth knowledge of theory and practical application of NMR with special focus on intrinsically disordered proteins and dynamic protein complexes using a number of theoretical and experimental NMR methods for their characterization. This will be done through lectures, and assigned literature readings. This course will also incorporate a problem-based learning approach, that requires reading assignments done outside the classroom, and in-class presentations.

Learning Resources: *S*elected journal articles, web resources such as http://www.cis.rit.edu/htbooks/nmr/, and http://www.nmrfam.wisc.edu/links/biochem-800/lecture_notes/

Understanding NMR Spectroscopy by James Keeler

Protein NMR Spectroscopy by Cavanagh, Fairbrother, Palmer, Rance & Skelton

Learner Outcomes: Students completing this course will:

- Acquire a basic understanding of:

  pulse sequences using product operator formalism,
  application of NMR to structure and dynamics of disordered proteins
  application of NMR to characterization of protein complexes
  recent NMR advances for analysis of large proteins,
  the potential and limitations of NMR for solving biological problems,
- Acquire hands-on experience in:
  NMR data analysis and interpretation,
  NMR hardware and software, data collection and processing,
  Protein structure determination and relaxation analysis software,

• Demonstrate an appropriate level of competence in the ability to apply, modify and/or create and contrast NMR experiments designed to qualitatively and/or quantitatively interrogate a wide variety of biochemical systems, and to evaluate the relative advantages and disadvantages compared to other techniques.
• Demonstrate the ability to produce quality critical analysis.

Targeted Learning Outcomes: The intention of the course is that by its end students will …

1. Understand how the basics of NMR pulse sequences.
2. Be able to design NMR experiments suitable for different systems.
3. Become familiar with processing multi-dimensional NMR spectra.
4. Become familiar with various NMR analysis strategies including those for protein structure determination, dynamics measurements and paramagnetic relaxation experiments.

Course Evaluation: Fulfillment of the student learning outcomes will be assessed through classroom presentations, one midterm, and a final exam project as follows:

Midterm (Thursday, February 4th)	35%
Paper presentations	35%
Project (Tuesday, March 15th)	30%

Course Outline:

Week 1 – Basic NMR spin physics, the vector model (Keeler, Chapters 1-4)

Week 2 – Data processing basics, Fourier transformation, Topspin (Keeler Chapter 5)

Week 3 – Product operator formalism: pulses, chemical shifts, and coupling (Keeler, Chapter 7)

Week 4 – Multi-dimensional NMR: COSY, HSQC, (Keeler Chapter 8)

Week 5 – Resonance assignments, Structure determination, Protein Interactions,

Week 6 – Relaxation, NOE (Keeler Chapter 9)

Week 7-8 – Dynamics, spectral density function, order parameters, protection factors, relaxation dispersion (15-min presentations)

Week 9– gradients, coherence selection (Keeler Chapter 11)

Week 10 – PRE, RDC and TROSY experiments (Keeler Chapter 10)

Final Project.

Presentations:
5-7 min except where indicated otherwise

Week 1: Larmor frequency, eigenvalue and eigenfunction, multiple quantum transitions vs single quantum transitions, on-resonance pulses,

Week 2: FID noise, sensitivity enhancement, resolution enhancement, zero filling, processing commands in TopSpin

Week 3: ----

Week 4: Explain using product operators a pulse sequence of your choice

Week 5: TALOS, CYANA, saturation transfer, other techniques for protein interactions (15 min presentations)

Week 6: ---

Week 7-8: application papers or theory of transverse relaxation, relaxation dispersion, chemical shift anisotropy, cross correlation, exchange broadening (15-20 min presentations)

Week 10: Application and theory of TROSY, RDC, PRE, (20 min presentation)

Final Project:
Research a completely new project with major health relatedness that can use NMR and be the basis of a research proposal. Write one page background presenting the gap in the knowledge, and two pages describing an NMR strategy you propose with focus on one major NMR experiment, how would the data be interpreted, and the reasons NMR is suitable for addressing this problem.