Overview and Study Guide for Protein NMR Lectures
Bio 5325, Fall 2006
D. P. Cistola

This block of lectures will introduce you to the multidimensional NMR methods used to study proteins. The main emphasis will be on the tools and strategies used to solve the three-dimensional structures of proteins and protein complexes in solution. Other aspects of biomolecular NMR, such as dynamics, nucleic acids and solid-state NMR, are not covered here but in Bio 5478 and other advanced electives offered through the Division and the Chemistry Department. More information on Bio 5478 is available at biochem.wustl.edu/courses.

Nuclear magnetic resonance is one of the most powerful and versatile methods available to study proteins. Yet, most biomolecular scientists know next to nothing about the method and hold gross misconceptions about its applicability. One reason is that the study of NMR can have a high energy barrier. However, this need not be the case. My role will be to lower your learning barrier and catalyze your comprehension. I will help you dodge all the non-essential details and focus on those basic concepts and strategies that you need to know at this point in your training. By the end of this course, you will be surprised at how well you understand this material!

In learning protein NMR, it is easy to get bogged down in complex spin physics or side-tracked with the bewildering array of pulse schemes. Conversely, an overemphasis on applications tends to leave one with a hollow view of the method – a view that shatters once new experiments are introduced. Therefore, it is important to be equally grounded in both theory and protein applications. The lectures of this course are divided accordingly. The first three NMR lectures address carefully chosen principles and pulse schemes leading up to HSQC – "the mother of most protein NMR experiments". The last three lectures address the experimental strategies used to solve protein structures by NMR. Mixed in are two additional lectures by Carl Frieden and Garland Marshall that address some interesting applications of NMR to protein folding and receptor-ligand interactions.

By the end of this course, you should be able to read an original research article describing a NMR structure of a protein, dissect and identify the experimental strategies and stages used to solve the structure, and evaluate the quality of the work. You should also understand the basic concepts and principles of NMR at the level taught in class. This includes an understanding of 2D HSQC: how it works and how it is used to study proteins.

<u>Lecture Notes and Online Resources</u>

My lecture and reading materials, problem sets and old exams are posted in two different locations:

1. the course website: dasher.wustl.edu/bio5325

2. the department website under Bio 5478:
   biochem.wustl.edu/courses/bio5478; see link at bottom for 5325

Because of copyright issues, the department/5478 site is password protected.
Username: webreathenmr
Password: 500600700

I will display my lecture notes on the screen during class. However, no hard copies of lecture handouts will be provided. If you wish to have a handout during class, please download and print a hard copy ahead of time.

Study Tips

Since NMR demands an iterative learning approach, I strongly urge that you review your notes and complete your reading prior to the next lecture. If you wait until the end of the semester to study, you will be overwhelmed!

The top priority in studying is to *understand the lecture notes*. I expect you to understand the material at the level taught in class. The second priority is to read the assigned papers and chapters given on the web site, using the guidelines given below. While you are welcome to consult any textbooks or review articles you like, I would recommend against pursuing materials beyond what I have assigned. I have carefully chosen articles and chapters that best match the level of material taught in this class. Other materials will tend to send you into topics tangential to those needed for this course and may just confuse you. The assigned material is challenging enough!

Optional Review Sessions and Office Hours

I will hold optional review sessions in order to work through practice problems and answer questions you may have from the lectures or reading material. Those sessions will be held in the 2807 North conference room across from my lab. You may come and go at any time during these periods:

Friday, November 3rd, 2 PM to 4 PM
Friday, November 10th, 2 PM to 4 PM
Friday, December 15th, 2 PM to 4 PM
Monday, December 18th, 2 PM to 4 PM

In addition, please contact me to arrange individual help with the material. The best way to reach me is by email at cistola@wustl.edu or you are welcome to stop by my office. I am difficult to reach by phone.

Comments About Specific Lectures

NMR I: make sure you understand the basic principles and parameters at the level I taught in class. Anything in the Evans chapter that goes beyond that level is less

important for now. Know what can be measured by NMR and what can be learned about the molecule from those measurements.

NMR II and III: Given a simple pulse sequence, you should be able to describe the state of the spin system using the vector and product operator descriptions. Refer to the problem sets or old exams for more examples. I strongly suggest that you work through practice problems, as this material becomes a lot easier with practice and familiarity. Be sure to know what a spin echo pulse sequence is and what it is used for. Know how polarization transfer is achieved and why it is important. Be familiar with the origin of the second dimension in NMR. It is vital that you understand the 2D HSQC pulse sequence, since you will encounter it many times in seminars and papers!

NMR IV and V: It is critical that you know the three (or four) stages used to solve a protein structure by NMR. Make sure you understand the different strategies for establishing resonance assignments: the types of samples used, the individual NMR experiments performed and the manner in which these experiments are put together to assign NMR peaks. Know how to map the secondary structure, especially using chemical shift and NOE restraints. Be familiar with the different types of NOESY experiments and the information content of each. Armed with this understanding, you should be able to sift through all the details presented in a NMR paper and identify the strategies used for each stage of structure determination.

NMR VI: Make sure you understand the approaches I discussed for characterizing ligand-protein complexes: chemical shift mapping and intermolecular NOEs. Know the difference between isotope-editing and –filtering, both with respect to the types of samples used and the NMR pulse sequences.

<u>Managing the Reading Material</u>

The readings go with the lectures as follows:

NMR I: Introduction to NMR (Evans chapter)
NMR II: Product Operator Formalism and Vector Model; Polarization Transfer (Freeman chapters)
NMR III: 2D NMR and HETCOR (Freeman chapters)
NMR IV: Omichinski et al. (example of assignment strategy 1);
Hill et al. (example of strategy 2);
Hodsdon et al. (example of strategy 3 and CSI);
Gardner et al. (example of strategy 4);
Kanelis 2001 (review of protein structure determination by NMR)
NMR V: Kanelis 2001 and Guntert 1998 (in-depth review of structure calculations from NMR data)
NMR VI: DiLello 2005 (chem. shift mapping); Fesik 1988 (isotope-editing);
Otting 1990 (isotope-filtering);
Tochtrop 2002 (binding energetics by NMR)

Some of the readings go beyond the level taught in lecture, so here are some suggestions for streamlining the reading material:

Evans chapter: pp. 35-42 covers solid-state NMR and is optional reading; pp. 50-53 is optional also

For NMR IV, I chose one original paper to highlight each of the four assignment strategies. Focus on the Methods sections to see how the different strategies were implemented. Also, note how Stages II and/or III of the structure determination process were carried out. Note how the results were reported.

The Kanelis 2001 review, one of the better ones I could find, discusses specific pulse sequences or details we did not cover in class. Don't get bogged down in any unfamiliar details. Just break things down as I suggest below.

The Güntert 1998 review discusses computational strategies used in Stage III. It is far more detailed than we need for this course, so just skim this article for general information on the different computational algorithms or to answer specific questions you may have. Don't worry about details beyond what is covered in class.

The DiLello 2005 was covered in class and is a nice example of a modern NMR paper: structure determination, chem. shift mapping, binding, etc. Good paper to read.

The Fesik 1988 Nature paper was one of the first describing isotope editing…. Although old, it provides a short and sweet description of the approach.

The Otting 1990 paper gives an example of isotope filtering in defining protein-DNA interactions…. Focus on Figure 2.

The Tochtrop 2002 paper is optional. It covers the determination of site-specific binding constants by NMR combined with ITC.

Dizzying Details

It is likely you will encounter some unfamiliar details in these papers, but don't get frustrated. The unfamiliar experiment is likely to be closely related to one you already know. Keep in mind that I have emphasized the overall strategies ("themes") and have given you some examples of specific experiments or pulse sequences that can be used. There are numerous individual experiments ("variations on the theme"), especially with assignment strategies 3 and 4 and the different flavors of NOESY experiments. Some papers will list specific experiments that I didn't talk about, such as 3D HCCH-TOCSY. When encountered with unfamiliar details, just consider the three stages of structure determination and ask yourself what strategies were used for each stage. An important clue is the type of isotope enrichment used. Also, note whether a specific experiment contains "NOESY" somewhere in the name. If it does, then it relies on through-space correlations between protons. If it does not have NOESY in the name, then it probably

relies solely on through-bond connections between various protons, carbons and/or nitrogens. Some NMR studies will use a combination of assignment strategies. For example, you might see that both ^{15}N- and ^{13}C/^{15}N-enriched proteins were used. In that case, the primary approach was assignment strategy 3, supplemented with some data from strategy 2. Remember that names such as “Strategy 1” and “Stage III” are my designations to help you categorize the information. You will not see such designations used explicitly in the literature.

Problem Sets

Problem Sets 4, 5 and 6 cover the NMR material. These problems are of the type you might expect on the closed-book final exam. I strongly suggest you work through them first without looking at your notes or the answer key. I may decide to post some more practice problems before the exam, so check the web site on a regular basis.

Exam Questions

I will likely ask you to analyze a pulse sequence using the vector and/or product operator models. I may ask you some conceptual questions about NMR fundamentals from lecture 1. It is highly likely that I will ask questions about protein structure determination strategies. I may give you an excerpt from a research paper describing the structure of a protein or ligand-protein complex determined by NMR. I may ask you specific questions about the experimental strategies used and what was learned about the system. I may ask you to evaluate the quality of the work given the guidelines we discussed in lecture. Previous exams and answer keys are provided on one or both of the web sites.

I hope you learn a lot from these lectures and develop a much greater appreciation for the NMR method and its applications to proteins!

Dave Cistola
10/24/2006