

POST ACQUISITION FILTERING: APPLICATIONS TO IN-CELL NMR

Alexander Krois

Major- Chemistry (BS, Biochemistry track)

Faculty Advisor- Dr. Gary Pielak
(Chemistry, Biochemistry)

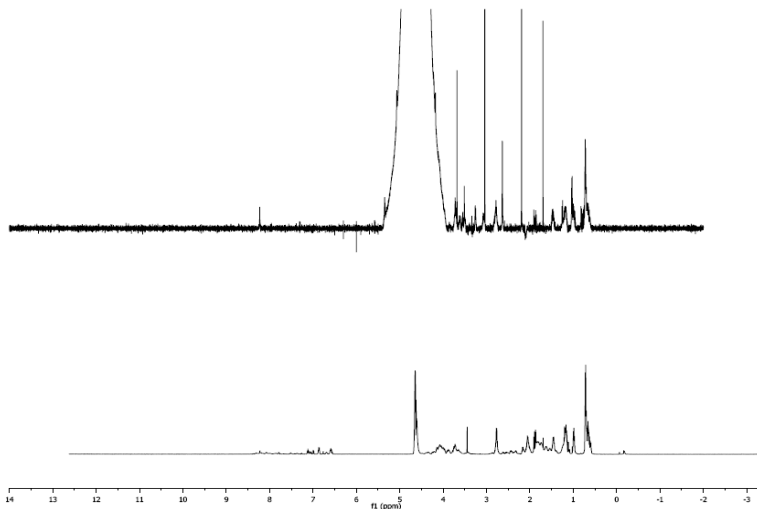
NMR Collaborator- Dr. Marc ter Horst
(Chemistry)

Graduate Advisor- Andrew Miklos
(Chemistry)



Background and Goals

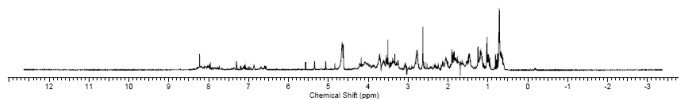
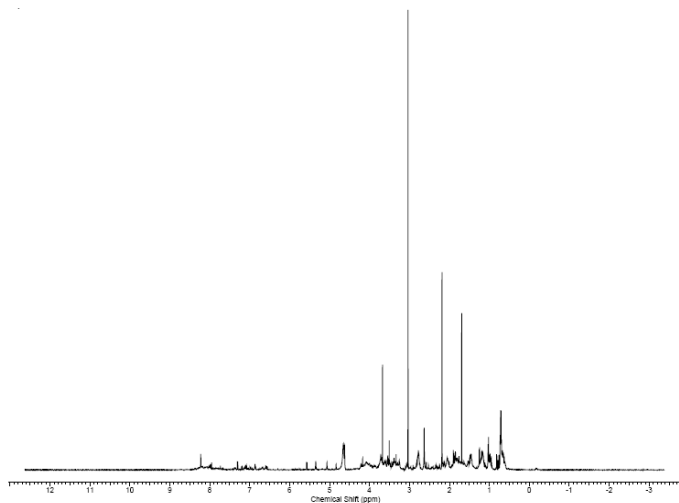
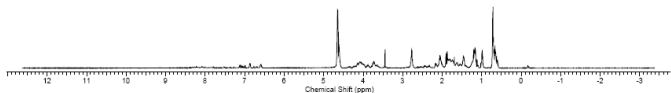
- Nuclear Magnetic Resonance Spectroscopy (NMR) is a powerful tool to nondestructively examine the structure, stability, and dynamics of molecules
- Understanding the structure, stability, and dynamics of proteins under physiological conditions is important in the study of diseases, yet most NMR is done in samples *in vitro* and not in cells
- When done in cells, NMR detects more than just the protein of interest (as a cell has metabolites, other proteins, etc.)
- The goal of my work is to develop a filtering and processing technique to remove unwanted, metabolite resonances



Top spectrum: purified α -synuclein with added metabolites and cell extracts (no water presaturation)

Bottom spectrum: just purified α -synuclein (with water presaturation)

Results



- I have developed a method to fit unwanted resonances, and then create a 2nd spectrum containing just the undesired signals
- The spectrum containing the undesired signals is subtracted from the original spectrum in the time domain (as FIDs) or in the frequency domain (as spectra), and so remove unwanted peaks
- As this subtraction method uses a 2nd spectrum or FID to alter the first, it should be possible to subtract the generated FID from signals shortly after they emerge from the magnet, enabling an increase in sensitivity
 - To develop the equipment needed to do this, a grant proposal has been sent to the NSF Major Research Instrumentation program ('Development of a Dynamic Range Extender for NMR Spectroscopy', PI's- Gary Pielak, Collin McKinney, Marc ter Horst); work accomplished here was included in the application

Top spectrum: purified α -synuclein (with water presaturation)

Middle spectrum: purified α -synuclein with added metabolites/cell extracts (with water presaturation)

Bottom spectrum: middle spectrum with major metabolite peaks subtracted through the use of a generated, 2nd spectrum/FID