A New “Babis” in Town

The NMR Community at the University of Minnesota is proud to welcome Professor Charalampos “Babis” Kalodimos to the University of Minnesota. He has joined the Department of Biochemistry, Molecular Biology and Biophysics, after spending time at Rutgers University in New Jersey as a Distinguished Professor in the Department of Chemistry and Chemical Biology. His work in NMR spectroscopy covers a wide range of biological phenomena and has used NMR as a means to investigate proteins and protein complexes exceeding 300 kDa. His most recent effort, published in Science, details his work with the large dynamic complex formed by trigger factor chaperone proteins and their substrate alkaline phosphatase. In this ground-breaking manuscript, Prof. Kalodimos reveals the molecular mechanism by which multiple trigger factor chaperone proteins recognize and bind large unfolded polypeptides, allowing proper folding and function. Even prior to this impressive achievement, the Kalodimos lab was well known for their work in attacking large protein complexes via NMR. Using state of the art NMR methodology, Prof. Kalodimos and his team have solved, arguably, the largest complex by solution NMR spectroscopy, the SecA-signal sequence. In light of Prof. Kalodimos’ achievements, his evident passion for the science of NMR spectroscopy, and his drive to push the boundaries of this technique, we consider ourselves very lucky indeed to have attracted such a promising investigator to our University.

Going Green

Helium Recovery System Soon to be Installed at MNMR
By Todd Rappe, Metabolomics Manager, MNMR Center

MNMR has two new major equipment purchases on the horizon. We have submitted an order for a new CryoProbe for the Bruker Avance 700-MHz spectrometer (7001). This Triple Resonance Inverse Detection TCI CryoProbe is equipped with cold 1H, 13C and 2H channels. The cryoProbe’s specifications include a 40% improvement on 1H sensitivity over the current TXI cryoprobe. As part of the purchase, we will also be upgrading the shim hardware which should result in a marked improvement in water suppression. We expect to have this new probe in early 2016.

We have also recently released a Request for Proposal (RFP) and initiated a project with Capital Planning for a helium recovery and liquefaction system for the NMR Center. Helium is a non-renewable resource. In recent years, political and manufacturing issues have put worldwide helium supplies at risk, resulting in shortages and cost increases. Currently MNMR purchases liquid helium to fill its instruments and, as the helium boils off, it is allowed to exhaust into the atmosphere and eventually into space. By recovering the helium, we will be able to lower operating costs, recycle a non-renewable resource and be buffered against future risks to supply. Stay tuned for more information on this project as it continues.
Amorphous solid dispersions (ASDs) are molecular mixtures of drug and polymer that can enhance oral drug absorption. However, the inherent physical instability leading to drug crystallization poses a major challenge. Our objective was to investigate the effect of different types of drug-polymer interactions (ionic and hydrogen bonding) on the physical stability of ASDs. We collaborated with Dr. Tata Gopinath from the Department of Biochemistry, Molecular Biology and Biophysics at UMN for his expertise in NMR technique to probe the molecular interactions in ASDs. Using $^{13}$C and $^{15}$N solid-state NMR spectroscopy, additional insights into drug-polymer interactions were obtained that were not discerned through infra-red spectroscopy. About 19 ppm shift in NMR spectra of ASDs (Figure below) provided evidence of strong H-bonding between N3 of ketoconazole (drug) and COOH group of PAA (ionically interacting polymer), which was confirmed by density functional theory calculations. An additional site of H-bonding was evident between N3 of ketoconazole and -OH of PHEMA (hydrogen-bonding polymer) in PHEMA ASDs (Fig. b). None of these changes in chemical shifts were seen in physical mixtures, indicating that the interactions were absent in physical mixtures but present in ASDs. Interestingly, a single spectroscopic technique could not completely characterize the drug-polymer interactions in ASDs. By combining IR and NMR spectroscopy, we obtained a comprehensive understanding of drug-polymer interactions. This research work was recently published in Molecular Pharmaceutics, 2015, 12 (9), pp 3339–3350.

Non-physiological homo-dimer structure for PqqD (PDBID: 3G2B)

**A New Spin on Kinase Research**

By W. Kaya Erbil, Dr. Nicolas Levinson’s lab, Department of Biochemistry, Molecular Biology, and Biophysics.

Kinases are allosteric enzymes central to signal transduction pathways that regulate growth and proliferation. As aberrant kinase signaling leads to cancer and existing inhibitors display poor selectivity, approaches for understanding protein dynamics are needed to provide new avenues to design selective kinase inhibitors for cancer treatments. A central paradigm in kinase inhibitor design has been to target the nucleotide-binding site by designing nucleotide analogs. These molecules are often promiscuous and fail in clinical applications because they grossly perturb the information flow through many different nodes in cell signaling pathways simultaneously. Inhibitors directed to sites other than the nucleotide-binding site based on general allosteric principles derived from our fundamental NMR studies may provide a new way to selectively target specific kinases in the cell, improving the precision and accuracy of cancer therapeutic design. Extensive X-ray crystallographic analyses of kinases free and bound to natural and synthetic ligands have revealed common patterns in the conformational transitions that underlie the activation pathway of these enzymes. However, they do not directly reveal the conformational energetic landscape of these molecules in solution. Understanding the nature of these energy landscapes is central to the rational design and development of selective allosteric inhibitors. Recent advances in the field of NMR have hinted that kinases are highly dynamic in solution, able to sample a manifold of distinct conformations in a particular state of enzymatic activity. We are obtaining spectra of evolutionarily related members of the kinase family in different states of activity, slowly revealing a set of complex, multilayered dynamic motions within the amide backbone and methyl side chains of the kinase domain. The interpretation of these spectra via assignment strategies will reveal sites amenable to pharmacological intervention with precisely guided allosteric inhibitors.
Proteins undertake a range of motions in terms of both time and distance scales. There are atomic vibrations on the subpicosecond time scale, picosecond to nanosecond backbone and side-chain fluctuations, millisecond conformational rearrangements, and slow breathing modes on the order of seconds (DD. Boehr et al, Chem. Rev. 2006, 106, 3055-3079). Time scales can cover from $10^{-15}$ to $>1$ sec. Any of these motions may be functionally significant and directly related to ligand exchange and/or catalysis.

Minnesota NMR Center (MNMR) has collected, developed, tested and improved many experiments to perform the study of protein dynamics in a wide time scale from subpicosecond to seconds. These experiments are complicated, but now NMR users can use python scripts to set-up these experiments:

1. ntl, nt2, mooe to setup backbone NH $^{15}$N $T_1$, $T_2$ and heteronuclear NOE experiments,
2. chd2 rl to setup methyl CHD$_2$ $^{13}$C $R_1$ and $R_{1p}$ experiments,
3. ch2d dtl to setup methyl CHD$_2$ & CH$_2$D $^2$H $R_1$ and $R_{1p}$ experiments,
4. htlrho and nt1rho to setup $^1$H and $^{15}$N $T_{1p}$ experiments,
5. hcpmg, ncpmg, ccpmg and chd2 depmg, and ch2d depmg to setup $^1$H CPMG of CHD$_2$, $^{15}$N CPMG, $^{13}$C CPMG of CH$_3$, $^2$H CPMG of CH$_2$ and CH$_2$D, respectively,
6. ncest, ch3_cest and chd2_cest to setup backbone NH $^{15}$N CEST and methyl CH$_3$ and CHD$_2$ $^{13}$C CEST, respectively,
7. hard to setup HARD (Heteronuclear adiabatic relaxation dispersion) experiments,
8. zzn and zczh3 to setup backbone NH $^{15}$N and methyl CH$_3$ $^{13}$C ZZ exchange experiments,
9. Dissolve lyophilized protein into D$_2$O and record a series of HSQC spectrum to monitor the H/D exchange,
10. ch3 mga to setup relaxation dispersion of $^1$H-$^{13}$C multiple quantum of methyl CH$_3$,
11. pre to setup $^1$H & $^{13}$C $T_1$ and $T_2$ experiments for PRE (paramagnetic relaxation enhancement).

As you can see, researchers can obtain backbone and side-chain dynamics information in a wide range of timescales.
Take a course with an NMR Scientist at the University of Minnesota MNMR Center

Undergraduate and Graduate students with an interest in NMR spectroscopy are able to join in specialized laboratory courses that allow hands-on experience with the instruments and teach various techniques. Courses in theory and NMR applications are also available. See listing below for NMR-related courses offered at the University of Minnesota. For more information or to register for classes, see http://onestop.umn.edu/.

BIOC 4225 - Laboratory in NMR Techniques (Offered May Session)
BIOC 5225 - Graduate Laboratory in NMR Techniques (Offered Spring Semester)
BIOC 5527 - Introduction to Modern Structural Biology (Offered Fall Semester)
BIOC 8001 - Biochemistry: Structure, Catalysis, and Metabolism (Offered Fall Semester)

Practical NMR Workshops

The MNMR Center offers three-day workshops focusing on the practical aspects of NMR, with topics ranging from sample preparation to instrument set-up and data processing. These workshops also serve as the training requirement for new users.

Please visit our web site at www.umn.edu/nmr/workshop.html for more information on course topics, dates and fees. These workshops are offered throughout the year. Contact Todd Rappe at rapp0006@umn.edu to reserve your spot in our next Practical Workshop.

Magnetic Moments

By Jonggul Kim

This last summer, Professor Burkhard Bechinger was an invited researcher from a joint project with the Minnesota NMR Center and the School of Dentistry. Professor Bechinger received his PhD from the University of Basel under the direction of Professor Joachim Seelig in 1989. He subsequently continued his training at the University of Pennsylvania under the Professor Stanley Opella until 1993. He is currently a Full Professor of Chemistry at the Université de Strasbourg. He was one of the first researchers to study the structure of membrane associated and bound biomacromolecules in their native environment using solid state NMR spectroscopy. His lab continues to study membrane biophysics using both oriented and magic angle spinning solid-state NMR, with a particular emphasis of elucidating the mechanism of anti-microbial peptides. While in Minnesota, Prof. Bechinger was involved in a collaborative study with Prof. Veglia and Prof. Sven-Ulrik Gorr from the School of Dentistry studying the mechanism of inhibition of the formation of biofilm by a novel anti-microbial peptide, GL13K.