NC STATE UNIVERSITY

Center for Comparative Medicine and Translational Research

CCMTR News

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Welcome, Dr. Kim Chappell!

On May 4, Dr. Kim Chappell joined the CCMTR as a clinical assistant professor to lead the Clinical Studies Core (CSC). Kim is an NC State alumna, receiving her BS in 1992 and DVM in 1997. She has spent the past nine years in clinical development within the veterinary pharmaceutical industry. She has led large, multi-site, companion animal effectiveness studies, has extensive experience with study design and protocol development, planning, budgeting, contracting, investigator recruitment, study initiation, study personnel training, data analysis and reporting, and FDA-CVM relations. The addition of Dr. Chappell will expand the services available through the CSC and provide faculty and corporate partners with a full-spectrum of assistance in clinical investigations.

A recently initiated phase 2 trial by Dr. Thierry Olivry (Allergy Research Core) offers a perfect example of the collaborative efforts the CSC intends to support. After initial studies performed by Dr. Olivry showed proof-of-principle for a compound to treat canine atopic dermatitis, Syntrix Biosystems, Inc. sought to further develop the compound. In collaboration with Dr. Olivry, Syntrix Biosystems was awarded a SBIR grant from the National Institute of Arthritis, Musculoskeletal and Skin Disease. Dr. Chappell and the CSC have been enlisted by Dr. Olivry to provide expertise and infrastructure to carry out the current trial, thus substantially reducing the time burden that would otherwise fall on the PI. "In recent years, the burden of running a large clinical trial in the middle of our regular receiving schedule became increasingly apparent," says Dr. Olivry. "The creation of the clinical studies core shall provide support to alleviate this burden and overcome previous hurdles. The expertise that Dr. Chappell brings to NC State will also help us raise our clinical trials to a higher level."

Development of the clinical investigations component of the CSC is supported by a $625,000 gift from Novartis Animal Health US, Inc. This program gives investigators the opportunity to evaluate novel approaches to diagnose and treat disease and deliver innovative solutions that enhance animal and human health.

For more information on the Clinical Studies Core: http://www.cvm.ncsu.edu/ccmtr/CSC.htm

Pilot Grant Research Symposium

The Pilot Grant Research Symposium was held May 14 at the CVM campus. The symposium highlighted research-in-progress talks from 2008 recipients of CCMTR pilot grants and CMTR Training Program fellows. Additional speakers included Dr. Bill Marzluff, a member of the CTSA executive committee. Dr. Marzluff gave an overview of the North Carolina Translational and Clinical Sciences Institute that was established to carry out the goals of the CTSA funding. He also provided an introduction to the programs available to CCMTR faculty (see below). Dr. Rob Whitehead from the NC State Office of Tech Transfer also gave an informative talk highlighting steps to protect and commercialize intellectual property. Dr. Whitehead encouraged investigators to engage his office early in the research process to avoid losing the opportunity to license or patent inventions.

NC TraCS (CTSA) Opportunities

The Center partnered with UNC-CH in a successful NIH Clinical and Translational Science Award. This is a very large grant that involves many research and clinical groups. One of the very significant benefits to Center members is access to pilot grant programs. There are now three pilot grant options available: 1) TraC$2K, $2,000 maximum, monthly submission deadlines; 2) TraC$10K, $10,000 maximum, submission deadlines every 3-4 months; and 3) TraCS Large Pilot Grant, up to $100,000, submission deadlines every 6 months. Dr. Jorge Piedrahita is the first CCMTR recipient of a TraCS pilot grant (10K) for his proposal entitled 'Generation of human hepatocytes in a transgenic pig'. Congratulations, Jorge!

How do you get the pilot grant money? Go to http://www.tracs.unc.edu/pilots.htm and review the pilot grant options and eligibility requirements. If you are interested in the $100K grants, please contact Dr. Gregg Dean to ensure your proposal is within the scope of the program and will be competitive. Please take advantage of this pilot grant opportunity!

New Trainees Selected for CMTRTP

Drs. Tracy Hill and Katie Sheats have been selected for the Comparative Medicine and Translational Research Training Program and will begin their program this summer.

Dr. Hill graduated from the University of Minnesota, St. Paul, MN, and is completing her residency in the Small Animal Internal Medicine, Clinician Investigator program at the CVM. Dr. Sheats graduated from NC State and is completing her residency in Equine Internal Medicine at the CVM.

These new trainees join Drs. Jaime Tarigo, Mike Wood, and Arnaud Van Wettere in the program. For details on the program: http://www.cvm.ncsu.edu/ccmtr/cmtrtp.html.

CCMTR Supports One Health Intellectual Exchange Group

The purpose of the One Health IEG is to foster interdisciplinary discussion and collaboration among students and scientists in the human, animal and environmental health sectors in the Triangle of North Carolina and beyond. The group was initiated by DVM students at the CVM who engaged representatives from UNC-CH and Duke. A broad-based steering committee was established and chaired by Dr. Prema Arasu. The group held four monthly meetings at the NC Biotechnology Center in RTP that featured several speakers from the CCMTR. Spring topics included "Autoimmune Skin Blisters - similar in humans and animals?" (Thierry Olivry, NC State CVM and Zhi Liu, UNC Medicine), "Stem Cells and Tissue Engineering" (Elizabeth Loboa, NC State Engineering), and "Climate and Disease" (Chris Woods, Duke Infectious Diseases and Fred Semazzi, NC State Meteorology).

Meetings will resume in the fall with Dr. Mac Law serving as chair of the steering committee. For more information contact Liz_Selisker@ncsu.edu. Meetings will be announced on the 'News and Upcoming Events' page of the website (http://cvm.ncsu.edu/ccmtr/News&Info.htm).

New Stem Cell Research Core

The CCMTR executive committee recently approved the establishment of the Stem Cell research core. Stem cells play an increasingly important role in regenerative medicine as well as cancer therapy and will likely revolutionize human and veterinary medicine. Clinical applications of stem cell research will have a significant impact in both veterinary and human medicine. The availability of large animal models will greatly facilitate translational research targeted toward human applications and will enhance our ability to form collaborations with other members of the North Carolina Translational and Clinical Sciences Institute. Dr. Jorge Piedrahita will lead this new core.

New Members

Dr. James Bonner, from the department of Toxicology (CALS), recently joined the Mucosal Pathophysiology research core. His research interests include the cellular and molecular mechanisms through which toxic environmental agents such as particles, nanoparticles, and metals cause fibroblast growth or matrix production resulting in pulmonary fibrosis.

Dr. David Threadgill, head of the department of Genetics (CALS) has joined the Oncology research core. His current research activities include the identification and functional characterization of alleles contributing to cancer susceptibility, the function of the *Erbb* gene family in development and disease, and the role of genetic variation in response to environmental stimuli.

We welcome James and David to the Center, and look forward to working with them.

Don't Forget to Designate the CCMTR in PINS!

Designation of the CCMTR in PINS is an important means for us to track the impact of the Center. If your proposal meets any of the following criteria, please select the CCMTR on the pull-down menu for 'Center': 1) the proposal is the result of a collaboration initiated through CCMTR activities, 2) CCMTR funding or a CCMTR service core was used to generate preliminary data, 3) the proposal will utilize a CCMTR service core, or 4) a CCMTR-supported trainee will be involved in the research. If you have any questions, please contact Liz Selisker.

CCMTR Promotion Encouraged

Increasing external recognition of the Center remains essential. Faculty members are reminded to acknowledge the Center in posters, presentations, publications, abstracts and grants. The Center logo is available for your use. Please contact Liz at liz_selisker@ncsu.edu or 515-8113.

Research Highlight: Dr. Robert Rose, Oncology Research Core

Dr. Rose, a member of the Oncology Research Core and an associate professor in the Department of Molecular and Structural Biochemistry, describes his research and provides helpful suggestions for CCMTR investigators interested in collaborating on a crystallographic project.

Structural biology can provide important molecular details about proteins implicated in disease, and facilitate the process of drug design. Many drug design companies, such as Glaxo-Smith-Kline, use crystallography as part of their drug design efforts. Crystallography therefore has the potential to contribute to translational research. My goal is to promote the application of protein crystallography as one tool in our effort to diagnose and treat disease. I will briefly describe how we use protein crystallography and small angle scattering in our lab to study transcriptional regulation. I will then mention some criteria that are important to consider when embarking on a protein crystallography project.

In my lab we study the insulin promoter as a model system for understanding the regulation of cell-specific transcription initiation. Insulin is expressed almost exclusively in pancreatic beta cells. As for many promoters, a combination of factors has been identified that are necessary for cell-specific expression of the insulin gene. We are using structural techniques to further characterize how these factors cooperate to achieve highly selective gene expression. In particular three factors are essential for transcription of the insulin promoter: the homeodomain protein Pdx1, the basic-helix-loop-helix (bHLH) factor E47/NeuroD, and a maf factor, MafA. Since none of these factors is expressed uniquely in beta cells, interactions among these factors are critical for restricting insulin expression to beta cells. Interestingly, the rat, mouse and human insulin promoters all bind these factors, but the spacing and number of binding sites differ. Interactions we are considering include direct contacts between these factors, cooperative DNA binding, co-binding with coactivators such as CBP, and chromatin modifications.

To date we have determined the crystal structures of the Pdx1 and E47/NeuroD DNA binding domains bound to DNA (Figure 1 and 2). These structures demonstrate one theme that I think is important for characterizing DNA binding specificity, and that is the context-dependency of the interactions. The Pdx1 structure showed two different conformations, indicating flexibility in how the homeodomain binds DNA depending on the DNA conformation. This may explain how Pdx1 can function with different transcription

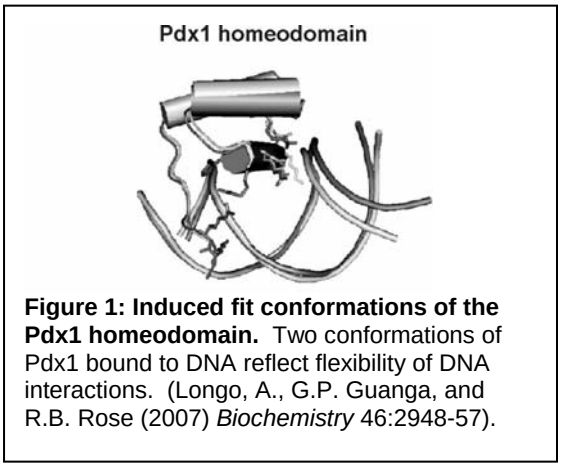
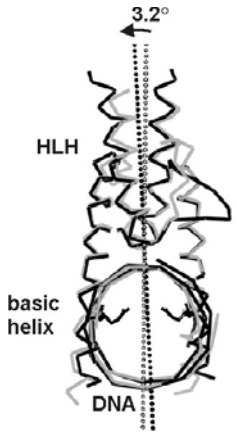


Figure 1: Induced fit conformations of the Pdx1 homeodomain. Two conformations of Pdx1 bound to DNA reflect flexibility of DNA interactions. (Longo, A., G.P. Guanga, and R.B. Rose (2007) *Biochemistry* 46:2948-57).

factor partners, for example during gut and pancreas development, and in the mature pancreatic beta and delta cells. The E47/NeuroD structure (Figure 2) showed that the heterodimer is oriented on the DNA, which is likely to be important for subsequent protein-protein interactions.



E47/NeuroD bHLH

Figure 2: "Wobble" of bHLH domains bound to DNA. We propose bHLH heterodimers orient on the DNA through a dynamic process in which the domain wobbles on the DNA to select the optimal position on the asymmetric CANNTG site. (Longo, A., G.P. Guanga, and R.B. Rose (2008) *Biochemistry* 47:218-29).

We are currently characterizing interactions between E47/NeuroD and Pdx1. One strategy for investigating complexes in the absence of crystals is by small angle scattering (SAS). This is a low resolution solution technique which can be used to generate a molecular envelope representation of large structures. The architecture of large complexes can be modeled from SAS data, particularly if crystal structures of the components are available.

Understanding how transcription factors function in different contexts will contribute to our ability to control the initiation of cell-specific transcription. For the treatment of diabetes, this will facilitate the generation of artificial beta cells for cell replacement therapy, derived for example from stem cells, pancreatic exocrine cells or liver cells. Mis-regulation of differentiation is also clearly important during oncogenesis. Cell-specific transcription factors, such as Pdx1 in beta cells or estrogen receptor in breast cancer cells, can regulate proliferation as well as differentiation. Targeting promoters that up-regulate proliferation may provide a cell-specific approach to treating cancer.

Practical considerations for starting a crystallography project:

Structural studies can take a lot of time, and therefore the targets must be chosen carefully. Generally granting agencies require proof that a sample will crystallize in order to fund a project. Often the time consuming steps for a crystallographic project are: 1) getting a pure, homogeneous, well-behaved sample, and 2) setting up crystallization trials. Crystallographic studies require large amounts (tens of milligrams) of sample. Proteins are typically expressed recombinantly in *E coli*, though some proteins require expression in eukaryotic cells to fold correctly. Often the full length protein contains disordered regions and sub-domains have to be found for crystallization. Once a well behaved sample is in hand, we typically screen about 300-400 conditions in our initial crystallization trials. The Biochemistry Department will soon purchase a crystallization robot, funded through a grant from the North Carolina Biotechnology Center. This will greatly speed crystallization trials and reduce the amount of sample required by a factor of 10 (the robot can set up 100 nl drops). The Department of Biochemistry has two home X-ray sources for data collection. In addition, NCSU has purchased time at a synchrotron beamline for high quality data collection, at Argonne National Labs in Chicago. Once a good diffracting crystal has been identified, solving the structure can be relatively quick (weeks to months), barring other difficulties. The structure solution and model building can all be done at NCSU.

CCMTR projects could benefit from crystallographic studies if the key players in a pathway have been identified and characterized. If groups have expressed proteins recombinantly, it may be worth trying to crystallize them. A picture of your favorite molecule can be very informative about function. As we believe in structural biology: function follows structure.