BIOCHEMISTRY & MOLECULAR BIOLOGY TODAY

MAY 2008 NO. 235

Chair's Message

A busy month indeed what with sign ups for teaching School of Medicine courses in the fall, graduations, departmental budget hearings and the departmental review. Our Texas summer also begins this month with the first days of temperatures above 90, to remind us of the hot humid months ahead.

I would like to remind our faculty about attending the Commencement exercises for the School of Medicine, which will be at Moody Gardens at 10:00 am on Saturday, May 31. We are maintaining the policy begun by Brad Thompson a long time ago providing for tenured/tenure track faculty to attend the SOM and GSBS commencement exercises, at the very least, every third year in order to provide an appropriate BMB presence. (Margie Wronski keeps track of this cyclical duty, and the department pays for the costs of gown rentals for those that do not have their own.) We appreciate the participation of all the faculty members scheduled to attend this year and we encourage others to join us in the faculty procession even if it's not necessarily your turn– faculty participation honors the students' achievements as well as the work of all our colleagues – the more the better.

Preliminary planning by the architect for the first phase of the Basic Science Building fifth floor renovation is underway. The main considerations are: providing space for new Bioinformatics faculty, renovating space for faculty already occupying the floor, and determining options for doing the renovation efficiently while minimizing disruption to faculty members' research.

Also, taking place during May will be first visits by faculty candidates for the position in the area of RNA signaling and regulation. Please make every effort to attend their seminars and feel free to share your evaluations of the candidates with the Chair or the recruitment committee chair, Werner Braun. This is an important area of research and I am personally very excited that we have the opportunity to complement our existing expertise in this area. If you wish to meet any of the candidates, please contact Margie Wronski and she will do her best to put you on the itinerary if possible.

I was very much intrigued by the talk given by Jim Goodwin, the recipient of the Graduate School Research Award, at the Graduate School commencement ceremonies. He focused his talk on the importance of communicating one's ignorance and celebrating one's failures; with the bottom line being the learning opportunities of the former and the need to try new ventures even when not assured of success. In my undergraduate training as an engineer at Cornell, we were encouraged to aim for 80% never 100%, - sort of the opposite of the perfectionist credo. The rationale is that it takes 50% effort to achieve that 80% accomplishment; the remaining 20% extracts another 50% of effort. A whole book, "In Search of Imperfection", was dedicated to this by the Nobel Laureate, Rita Levi-Montalcini.

The persistent pressures for teaching our students and still accomplishing our achieving research aims together with our service obligations would suggest that we should work harder and more efficiently, I think it is equally important to reserve time for thinking - not just reading that new Science article in haste but actually spending a little time thinking about our projects from a long term perspective. Just as we are now embarking on developing a five-year plan for the department and the Research Executive Committee will soon begin a process to develop the next five-year strategic research plan for UTMB, this summer might be a good time for each of us to think about our personal five-year strategic plans, as we enjoy the somewhat slower rhythms of summer on our sub-tropical island.

-regino

Inside this issue:

Special Items of Interest

Graduate Program News

BSCB graduate student, Kurtis Anderson, has just been appointed a Keck Training Fellow in the Computational and Structural Biology in Biodefense Training Program. Congratulations to Kurtis for being awarded a fellowship in his first year in graduate school- a very unusual occurence.

Kurtis' research project title is: "Next-generation thioaptamers as treatment for viral hemorrhagic fever".

- Debora Botting

Awards and Announcements

Grants:

Dr. Catherine Schein received a Mitchell Award at the end of March.

Dr. Heidi Spratt received a K25 Career Development Award, “Biomarker Discovery for Hepatitis C Progression using Machine Learning Techniques“ which will be funded for 5 years.

Dr. Olivera Nesic received a Sealy Grant

An European Union Patent was filed:

Bruce A. Luxon, Mahvash Tavassoli, Anoma Somasunderam, Marcella Flinterman, David Gorenstein, “Thioaptamer targeted to the EGF-Receptor”. Prof. Tavassoli and Dr. Flinterman are at King’s College London. This work was supported by a grant from the UK Dept. Trade and Industry to M. Tavassoli and B. Luxon.

The Sealy Center for Structural Biology and Molecular Biophysics held its 13th Annual Structural Biology Symposium May 16-17, 2008 at UTMB, chaired by Drs. Vincent Hilser and Robert Fox. Professor Tom Steutz (Yale, HHMI) gave the keynote address: “The Structural Basis of Crick's Central Dogma”. Other speakers included: Arthur Johnson (TAMU), Jose Barral (UTMB), Leemor Joshua-Tor (CSH), Susan Marqusee (UC Berkeley), David Agard (UCSF, HHMI), Yousif Shamoo (Rice), Tracy M. Handel (UCSD), Dagmar Ringe (Brandeis), Thomas Walz (Harvard), and William E. Balch (Scripps). Three graduate students were selected to present short talks: Ms. Mikyung Han (Baylor), Mr. Kerry Fuson (UTMB), Mr. Char Hu (UH). The meeting concluded with a banquet at The Hotel Galvez.

Overall, almost 270 students, staff and faculty from as far away as Germany attended the two day event.

Next year’s 14th Annual Symposium will be March 27-28, 2009, mark your calendars now.

Research Spotlight: Aptamer Development Laboratory

The Aptamer Development Laboratory was started in December 2006 with funding from the Sealy Center for Molecular Medicine and the Dean of the School of Medicine. Steve Widen was recruited to establish this program and produce aptamers.

Aptamers are single strand DNA or RNA molecules that fold into structures that allow binding to other molecules. Aptamers have been isolated that bind to inorganic and small organic molecules, antibiotics, other nucleic acids, peptides, proteins, carbohydrates, complex mixtures and even viruses and whole cells. Aptamers may bind with an affinity similar to antibodies and have several advantages over antibodies such as *in vitro* selection and the ability to be synthesized chemically. Modifications to the nucleotides can increase affinity and stability. An aptamer to VEGF has been approved by the FDA for treatment of Macular Degeneration.

In nature, RNA aptamers that recognize small molecules are used to regulate gene expression. Called riboswitches, these aptamers are part of the messenger RNA sequence and may regulate transcription rates, termination and translation, depending on binding of the small molecule. This type of regulation is also of interest to the synthetic biology field to create new mechanisms for controlling gene expression.

Aptamer selection typically starts with a library of up to 10^{15} molecules. Whether RNA or DNA, the molecules consist of a randomized region of 20 or more bases flanked by constant regions that allow for PCR amplification of the molecules. In order to select protein specific aptamers, the protein is incubated with an aptamer library to allow binding, then the protein bound DNA or RNA is separated from the unbound material. Once separated, the bound material is amplified by PCR followed by single strand purification (DNA), or RT-PCR followed by *In vitro* transcription (RNA). This material is used for the next round of selection, and typically more than a dozen rounds are needed to identify specific aptamer sequences.

Probably the most common separation method utilizes filter binding of the protein, but gel electrophoresis, column chromatography, bead binding and selection and other methods are also used. Since there may be just a few active sequences in the 10^{15} molecule library, efficiency of separation is critical. Capillary electrophoresis has been demonstrated to be a very efficient separation method and we have a Beckman P/ACE MDQ system dedicated for this purpose.

We have used filter binding, affinity chromatography and bead based separations to isolate aptamers binding to IKKγ and IκB, two proteins in the NFκB pathway. One aptamer for IKKγ has an affinity in the low nanomolar range. Functional tests are being set up to determine if it has any inhibitory effect on IKKγ activity. We are in the process of isolating aptamers for several other proteins and initiated a project to isolate aptamers to small molecules.

For more information, contact Dr. Tom Wood or Dr. Steve Widen.

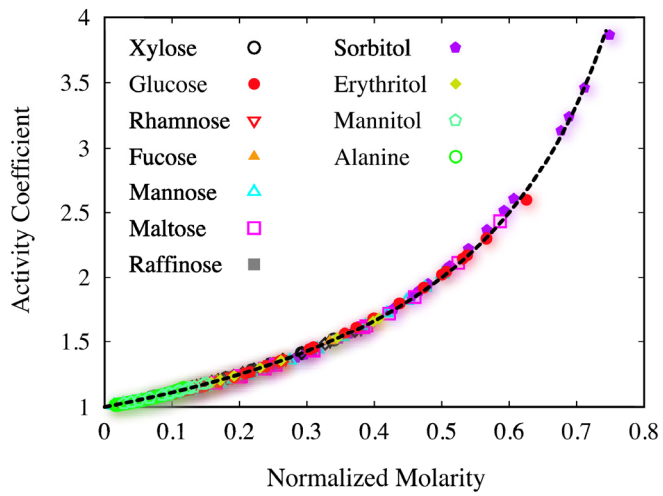
Faculty Focus: Jörg Rösgen, Ph.D.

Jörg Rösgen received both undergraduate and graduate degrees from the University of Münster in Germany, with majors in Biology and Physical Chemistry. He joined UTMB in 2001 as a postdoctoral fellow, and worked as a Keck fellow at UTMB and University of Houston. Since 2005 he has been research assistant professor at the Department of Biochemistry and Molecular Biology, and he joined the graduate faculty in 2007.

The cytosol is a highly crowded environment in which molecules are assumed to behave very differently compared to the dilute solutions that are normally used in quantitative biochemistry. Under the crowded cellular conditions it is not permissible to use concentrations in equilibrium and kinetic equations – chemical activities have to be used instead. It is generally recognized that macromolecular crowding should have a major impact on the chemical activities of larger biomolecules. Much less attention is paid to the effects of small molecules on macromolecules, and virtually nothing is known about the chemical activities of small molecules under crowded non-ideal conditions.

My long term goal is to understand both the magnitude and the origin of chemical activities of biomolecules under conditions resembling the crowded cytoplasm. This involves developing new experimental techniques to surmount throughput barriers in current approaches. It also involves developing solution theories to understand the collected data.

My current research focuses on osmolytes and their solvation interactions with other molecules. Osmolytes are small organic molecules that naturally occur in virtually all organisms – often at concentrations in the range of around one to several molar. Osmolyte concentrations can strongly fluctuate, because these molecules are used to counteract variable cellular stresses. Investigating the effect of changing osmolytes concentration on other biomolecules is thus of direct biological importance, especially in disease states in which osmolyte regulation is disturbed.



Hyperbolic dependence of osmolyte chemical acvity on its concentration. Osmolyte thermodynamic behavior is amazingly uniform and simple.

Recent results form our lab suggest that beyond the known effects of osmolytes on macromolecules, these small compounds have an even stronger effect on metabolites and small signaling molecules. The implications for cell homeostasis could be significant. Besides such phenomenological findings we are also interested in the physics behind this behavior. We were able to show that osmolytes mediate their effects through an unexpected solvation behavior in which changes in osmolyte solvation are always accompanied by a parallel and equal change in hydration. As a consequence, the polynomial that contains all the thermodynamic information of the system (the so-called partition function) reduces to a simple linear function, and all thermodynamic equations become exceptionally simple.

Work in the near future will focus on the development of high-throughput instrumentation appropriate for investigating osmolyte mixtures and their effect on biochemical reactions.

BMB Faculty Publications

Choi KH, McPartland J, Kaganman I, Bowman VD, Rothman-Denes LB, Rossmann MG (2008) Insight into DNA and protein transport in double-stranded DNA viruses: The structure of bacteriophage N4. *J. Mol. Biol.* 378 (3): 726-36.

Bao X, Sinha M, Liu T, Hong C, **Luxon BA,** Garofalo RP, Casola A., "Identification of human metapneumovirus-induced gene networks in airway epithelial cells by microarray analysis.", Virology. 2008 Apr 25;374(1):114-27. Epub 2008 Jan 29.

Kang J, Lee MS, Copland JA 3rd, **Luxon BA, Gorenstein DG.,** "Combinatorial selection of a single stranded DNA thioaptamer targeting TGF-beta1 protein.", Bioorg Med Chem Lett. 2008, Mar 15;18(6):1835-9. Epub 2008 Feb 13.

Yang, Xianbin, Beasley, Duane, Engelhardt, Johnnie, Shumbera, Mark, **Luxon, Bruce A.** and **Gorenstein, David** G., "Bead-Based Approaches to Develop Thioaptamers for Diagnostics and Therapeutics", Phosphorus, Sulfur, and Silicon and the Related Elements, 2008 May 6, 183:2, 469-472.

Anil K. Mantha, Numan Oezguen, **Kishor K. Bhakat,**Tadahide Izumi, **Werner Braun** and **Sankar Mitra.** Unusual Role of a Cysteine Residue in Substrate Binding and Activity of Human AP-Endonuclease 1, J. Mol. Biol. (2008) 379, 28–37

Atasheva S, Garmashova N, Frolov I, **Frolova E**. Venezuelan equine encephalitis virus capsid protein inhibits nuclear import in Mammalian but not in mosquito cells. J Virol. 2008 Apr;82(8):4028-41. Epub 2008 Feb 6.

Gorchakov R, **Frolova E**, Sawicki S, Atasheva S, Sawicki D, Frolov I. A new role of ns polyprotein cleavage in Sindbis virus replication. J Virol. 2008 Apr 16. [Epub ahead of print]

Amira Zaky, Carlos Busso, Tadahide Izumi, Ranajoy Chattapadhyay, Ahmad Bassiouni, **Sankar Mitra** and **Kishor K. Bhakat.** Regulation of the human AP-endonuclease (APE1/Ref-1) expression by the tumor suppressor p53 in response to DNA damage. Nucleic Acids Res. 2008 Mar;36(5):1555-66. Epub 2008 Jan 21.

Mantha AK, Oezguen N, **Bhakat KK**, Izumi T, **Braun W, Mitra S**.Unusual role of a cysteine residue in substrate binding and activity of human AP-endonuclease 1. J Mol Biol. 2008 May 23;379(1):28-37. Epub 2008 Apr 3.

Ye Y, Martinez JD, **Perez-Polo JR**, Lin Y, Uretsky BF, **Birnbaum Y**. The role of eNOS, iNOS and NF{kappa}B in upregulation and activation of cyclooxygenase-2 and infarct size reduction by atorvastatin. Am J Physiol Heart Circ Physiol. 2008 May 9. [Epub ahead of print].

Mark Andrew White, Natalia Mast, Ingemar Bjorkhem, Eric F. Johnson, C. David Stout, and Irina A. Pikuleva, The Use of Complementary Cation and Anion heavy-atom salt derivatives to solve the structure of cytochrome P450 46A1, *Acta Cryst. D* 2008; **65**(16);487-95 **EPUB**: April 20.

To have your travels included in the monthly newsletter, please send the information directly to Lisa Pipper (lpipper@utmb.edu) by the 1st of each month.

Faculty on the Road

Dr. Satish K. Srivastava presented at the 2008 Association for Research in Vision and Ophthalmology (ARVO) meeting in Ft. Lauderdale, Florida on April 28th thru April 30th

Dr. Kota V. Ramana was a reviewer for the American Heart Association Region III Review Study Section in Los Angeles, California on April 17th thru April 18th.

He also attended and presented at the 2008 Association for Research in Vision and Ophthalmology (ARVO) meeting in Ft. Lauderdale, Florida on April 28th - 30th.

Dr. Lillian Chan attended the American Society for Gene Therapy Annual Meeting in Boston, Massachusetts on May 29th thru June 3rd.

Dr. Krishna Rajarathnam participated in the American Heart Association South Central and Western States Peer Review Committee in Los Angeles, California on April 15-16th.

Dr. Cheryl Watson was an invited speaker at a seminar at Drexel University School of Medicine Institute for Women's Health in Philadelphia, PA on April 23-25th.

ADMINISTRATOR'S NOTES

THEY'RE ALMOST HERE… STATE FIRE INSPECTORS TO ARRIVE THE WEEK OF JUNE 16

UTMB has been informed that the State Fire Inspectors are expected to be on campus for several days in the week on June 16, although there is some possibility they could arrive earlier. BMB labs and other areas have done a good job checking on compliance with fire safety requirements. As the date for the inspection approaches, please be sure to keep things in appropriate shape:

- Materials stored on shelves must be at least 18 inches from the ceiling.
- Aisles and exits must be kept clear.
- Fire-rated doors must not be propped open.
- Flammable materials must be properly labeled and stored appropriately.

EFFORT CERTIFICATION DEADLINES HAVE BEEN EXTENDED

The new deadline for "primary individuals" to complete effort confirmation for themselves and any researchers working on projects for which they are the PI is now

MAY 31, 2008. Effort is to be confirmed for the period of September 2007 through February 2008. If there are any questions, please contact TK Kirtley, the Lead Effort Coordinator for BMB at tkirtley@utmb.edu.

ELEVATOR PROBLEMS IN MRB and BSB

We are keeping up regular communication with FOAM managers Lewis Cantrell and David Ketchens regarding the persistent elevator problems Department members are experiencing. In the next couple of months, non-working indicator lights on call buttons, floor buttons, and direction signals will be replaced. We will be notified when the parts have arrived and work on this project has begun. Dave Ketchens has asked to attend a BMB faculty meeting to discuss the elevator problems and the solutions that are being pursued. He will be included on the agenda for either the June or the July meeting.

Best wishes to everyone for a safe and enjoyable holiday weekend.

-Marianne

Featured Abstract by BMB Faculty

Insight into DNA and protein transport in double-stranded DNA viruses: the structure of bacteriophage N4.

J Mol Biol. 2008 May 2;378(3):726-36. Epub 2008 Mar 4

Choi KH, McPartland J, Kaganman I, Bowman VD, Rothman-Denes LB, Rossmann MG.

Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX 77555-0647, USA.

Bacteriophage N4 encapsidates a 3500-aa-long DNA-dependent RNA polymerase (vRNAP), which is injected into the host along with the N4 genome upon infection. The three-dimensional structures of wild-type and mutant N4 viruses lacking gp17, gp50, or gp65 were determined by cryoelectron microscopy. The virion has an icosahedral capsid with T=9 quasi-symmetry that encapsidates well-organized double-stranded DNA and vRNAP. The tail, attached at a unique pentameric vertex of the head, consists of a neck, 12 appendages, and six ribbons that constitute a non-contractile sheath around a central tail tube. Comparison of wild-type and mutant virus structures in conjunction with bioinformatics established the identity and virion locations of the major capsid protein (gp56), a decorating protein (gp17), the vRNAP (gp50), the tail sheath (gp65), the appendages (gp66), and the portal protein (gp59). The N4 virion organization provides insight into its assembly and suggests a mechanism for genome and vRNAP transport strategies utilized by this unique system.