BIOCHEMISTRY & MOLECULAR BIOLOGY TODAY

FEBRUARY 2007 NO. 222

UTMB

BMB Biochemistry & Molecular Biology

Chair's Message

Now that we have our first round of faculty recruitment completed, we are beginning a second round. Advertisements in *Science* and *Nature* will soon appear and a new Faculty Recruitment Committee has been assembled for the task ahead. You will hear more details once the initial group of candidates is announced. Basically, we are recruiting for three junior faculty positions in partnership with the George P. and Cynthia Woods Mitchell Center for Neurodegenerative Diseases, the Sealy Center for Molecular Medicine and the Moody Center for Research in Head and Spinal Cord Injury. We will keep you informed.

Over the next couple of months, the faculty at large will be discussing the Faculty Compensation Plan for next year. I charged a committee, Chaired by Ana Pajor, with the task of revising our plan with a strong focus on tenure track faculty salary stability. A draft has been circulated to the faculty for discussion at faculty meetings and I am confident that we will arrive at a useful instrument. We will examine this document on an annual basis to keep it current with existing challenges and opportunities. One outcome to date that is likely to be adopted is the concept of basing compensation on three year averages of performance. Other Basic Science Chairs have found this idea attractive and a group of representatives from the Basic Science Departments will discuss the idea of making this uniform across these Departments. The Basic Science Departments have met with the Dean of Medicine to reach some consensus on salary recovery expectations to provide a more even playing field across these Departments. I have also charged another group to address the status of non-tenure track faculty in terms of the three missions of the Department: research, teaching, and service. John Wiktorowicz is chairing this group.

For the second year, the Molecular Basis of Disease BMB graduate course is a success! As you might remember, last year Vince Hilser, John Papaconstantinou and Claudio Soto ran a course focused on protein misfolding in disease processes, with distinguished visitors sharing their expertise with our students, not to mention the faculty. This year we were able to incorporate the course into the Mary Huling Edens Memorial Lecture Series to allow us to continue this outstanding course. We have to thank Wayne Bolen, Olivera Nesic and John Papaconstantinou for their leadership in designing and running the course. This is the kind of translational effort in the teaching arena that is central to our goals for the department and the school.

As we have stated before, we are developing an in-house study section for the benefit of the faculty. We are also trying to optimize the process of grant submission to enhance the opportunities of the faculty during a difficult time for NIH funding. To that end, if you are planning to submit a grant at the next cycle or if you would like some form of critique to aid in the development of a research project, please let our Vice-Chairs or myself know so we can make arrangements to allow you the utmost flexibility and opportunity.

I am very pleased to announce that Jeffrey P. Rabek, Ph.D., has been appointed as Assistant Dean for Student Affairs and Admissions for the School of Medicine effective February 1, 2007.

Congratulations.

Inside this issue:

Special Items of Interest

Graduate Program News—BCSO News

This month, the BCSO has planned to organize and participate in many activities. First, the BCSO participated as volunteer science judges at the Galveston County Science & Engineering Fair on the Mitchell campus of Texas A&M at Galveston on Feb 3, 2007. A group of BCSO students attended the science fair and judged in various categories such as biochemistry, physics, computer science, math, and environmental sciences . On Feb 20th, we are organizing a BCSO rock climbing session at the League City Rock Climbing Gym. We are also organizing group trips to Houston to watch the Houston Rockets - San Antonio Spurs game on Saturday March 3 and to a Cirque du Soleil Corteo show on Saturday April 28. Please come out and join us.

Jason Vertrees, BSCB 3rd year working at Dr. Hilser's cluster.

Awards and Announcements

Dr. Vincent J. Hilser was invited to become a standing member of the International and Cooperative Projects (ICP1) study section at the Center for Scientific Review, National Institutes of Health

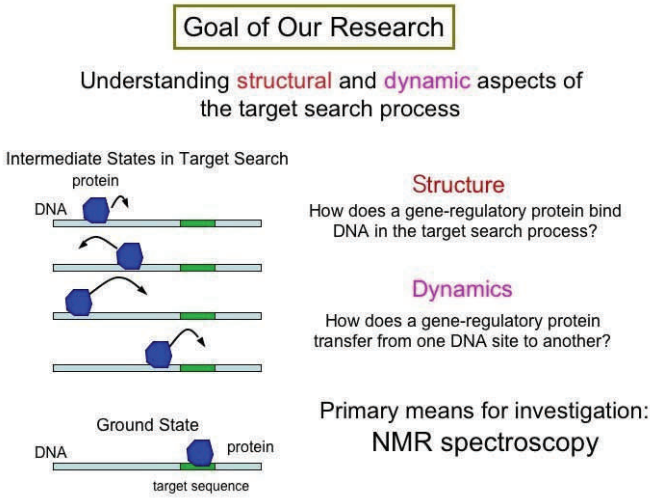
The ICP1 study section reviews applications for the Fogarty International Research Collaboration Award (FIRCA) in basic biomedical science under the R03 small grant mechanism. The award facilitates collaborative basic biomedical research between scientists supported by NIH and investigators in developing countries of Latin America, Asia, Eastern Europe, Russia and former Soviet Union Republics. This award is supporting discrete, well-defined projects that realistically can be completed in three years and that require limited levels of funding. Because the research plan is restricted to 10 pages, a small grant application will not have the same level of detail or extensive discussion found in an R01 application.

Dr. Brad Thompson will be one of two Faculty Advisors for the 2007 National Student Research Forum, to be held on April 26 and 27. The Research Forum is planned and organized by students in the School of Medicine. The 2007 Forum marks the 48th year of UTMB's sponsorship of the event. More information is available at the Forum website at www.utmb.edu/nsrf/.

Faculty Focus: Junji Iwahara, Ph.D.

Dr. Junji Iwahara received his Ph.D. from the University of Tokyo (Japan) in 1998. His graduate work focused on the investigation of interactions between centromere proteins and DNA using NMR spectroscopy. Since then, his research has involved study of the biophysics of protein-DNA interactions by means of NMR. He joined Robert T. Clubb's laboratory at UCLA in 1998 as a postdoctoral fellow and then moved to G. Marius Clore's laboratory at NIH/NIDDK in 2002 as a research fellow. He has developed various new NMR methods for investigation of protein-DNA interaction. Dr. Iwahara joined BMB as an Assistant Professor in January 2007.

A macromolecular binding event is not simply a two-state exchange between free and bound states. In fact, previous kinetic investigations have suggested that the process of the specific complex formation goes through intermediate states called target searches or encounter complexes, and the presence of such intermediates greatly accelerates the overall process. In terms of structure, how do individual components bind to each other at the intermediate states? How dynamic are they? These are fundamentally important questions for understanding macromolecular recognition, but structural biology has not provided adequate answers yet. Because of the very low population at equilibrium and the dynamic nature of intermediate states, investigation of these questions is extremely difficult. We have developed a number of powerful NMR methods that permit characterization of the intermediates in macromolecular binding at equilibrium. Applying the new techniques, we investigate structural and dynamic aspects of binding intermediates. Currently, the focus in our research is on the target search process whereby gene-regulatory proteins are able to efficiently and rapidly locate their specific DNA target sequence in a sea of non-specific DNA.



Selected publications (5 out of 28)

1. Iwahara, J., Clore, G.M. (2006) Detecting transient intermediates in macromolecular binding by paramagnetic NMR. Nature 440, 1227-1230.
2. Iwahara, J., Zweckstetter, M., Clore, G.M. (2006) NMR structural and kinetic characterization of a homeodomain diffusing and hopping on nonspecific DNA. Proc Natl Acad Sci U S A 103, 15062-15067.
3. Tang, C., Iwahara, J., Clore, G.M. (2006) Visualization of transient encounter complexes in protein-protein association. Nature 444, 383-386.
4. Iwahara, J., Clore, G.M. (2006) Direct observation of enhanced translocation of a homeodomain between DNA cognate sites by NMR exchange spectroscopy. J Am Chem Soc 128, 404-405.
5. Iwahara, J., Jung, Y.S., Clore, G.M. (2007) Heteronuclear NMR spectroscopy for lysine NH3 groups in proteins: Unique effect of water exchange on 15N transverse relaxation. J Am Chem Soc 129, in press.

Spotlight:
Molecular Genomics Core

The mission of the Molecular Genomics Core (MGC) is to provide technical expertise in the areas of gene expression analysis and gene mapping to UTMB investigators that will enhance the successful attainment of their research goals. The MGC offers technical support for gene expression analysis using Affymetrix Gene Chips as well as other commercially available arrays. Validation of expression analysis using real-time quantitative RT-PCR is available using either TaqMan® or SYBR® I Green assays. Both chemistries are available for either absolute or relative quantitation. Please see our website for further explanation of both the chemistries and quantitations (www.scmm.utmb.edu/genomics/realtime.htm).

SYBR® is a cost effective alternative for those investigators who wish to perform expression analysis on a number of genes which makes TaqMan® chemistry cost prohibitive. SYBR® is a highly specific, double-stranded DNA binding dye, used to detect PCR product as it accumulates during PCR cycles. The most important difference between the TaqMan® and SYBR® chemistries is that the SYBR® chemistry will detect all double-stranded DNA, including non-specific reaction products. A well-optimized reaction is therefore essential for accurate results.

Background

Small molecules that bind to double-stranded DNA can be divided into two classes: intercalators or minor-groove binders. SYBR® is an intercalator like ethidium bromide, whereas TaqMan® is a minor-groove binder. Regardless of the binding method, there are two requirements for a DNA binding dye for real-time detection of PCR: increased fluorescence when bound to double-stranded DNA and no inhibition of PCR. The conditions under which the reactions are carried out permit the use of the SYBR® without PCR inhibition and increased sensitivity of detection compared to ethidium bromide.

Assay Design

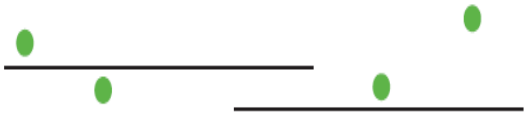
Because SYBR® binds doubled stranded DNA regardless of sequence, primer design and rigorous validation is critical. Whenever possible, the assay is designed so that one of the two primers transverses an exon-exon junction. This design technique eliminates contribution from contaminating genomic DNA (gDNA) found in most RNA samples. Furthermore, when necessary we DNase treat all RNA samples to further eliminate any gDNA contribution. Once the real-time assay is complete, we perform a dissociation curve to eliminate the possibility of multiple PCR products which would confound the results. In the rare event that multiple species are found, the assay is re-designed.

To learn more about the Molecular Genetics Research core, please visist www.scmm.utmb.edu/genomics/

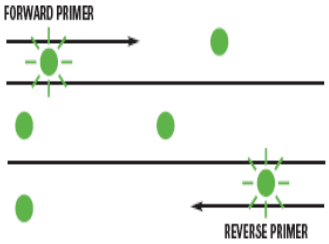
How the SYBR Green I Dye Chemistry Works

1. Reaction setup: The SYBR® Green I Dye fluoresces when bound to double-stranded DNA.

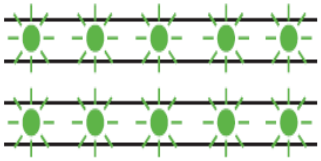
2. Denaturation: When the DNA is denatured, the SYBR® Green I Dye is released and the fluorescence is drastically reduced.



3. Polymerization: During extension, primers anneal and PCR product is generated.



4. Polymerization completed: When polymerization is complete, SYBR® Green I Dye binds to the double-stranded product, resulting in a net increase in fluorescence detected by the 7900HT system.



Publications & Grant Awards

Tammali R, **Ramana KV** and **Srivastava SK** (2007). Aldose reductase regulates TNF-a-induced PGE2 production in human colon cancer cells. Cancer Letters (In Press).

Ramana KV, Reddy ABM, Tammali R and **Srivastava SK** (2007). Aldose Reductase Mediates Endotoxin-Induced Production of Nitric oxide and Cytotoxicity in Murine Macrophages. Free Radical Biology & Medicine (In Press).

Rossmann, M.G., Arisaka, F., Battisti, T.J., Bowman, V.D., Chipman, P.R., Fokine, A., Hafenstein, S., Kanamaru, S., Kostyuchenko, V.A., Mesyanzhinov, V.V., Schneider, M.M., **Morais, M.C.,** Leiman, P.G., Palermo, L.M., Parrish, C.R., & Xiao, C. (2006) "From Structure of the complex to understanding of the biology" Acta Crystallogr D Biol Crystallogr. Jan;(63)(Pt 1) 9-16.

Yochai Birnbaum, Yumei Ye, Yu Lin, Sheldon Y Freeberg, Ming-He Huang, Jose R Perez-Polo, Barry F. Uretsky. Aspirin augments 15-epi-lipoxin A4 production by lipopolysaccharide, but blocks the pioglitazone and atorvastatin induction of 15-epi-lipoxin A4 in the rat heart. Prostaglandins & Other Lipid Mediators 83:89-98, 2007.

Das A, Wiederhold L, Leppard JB, Kedar P, Prasad R, Wang H, Boldogh I, Karimi-Busheri F, Weinfeld M, Tomkinson AE, Wilson SH, **Mitra S**, **Hazra TK**. 2006 NEIL2-initiated, APE-independent repair of oxidized bases in DNA: Evidence for repair complex in human cell. DNA Repair: 5: 1439-48.

Oezguen, N., **Schein, C.H.,** Peddi, S.R., Power, T.D., Izumi, T. and **Braun, W.** A "moving metal mechanism" for substrate cleavage by DNA repair endonuclease APE-1. Proteins, (in press) 2007.

Schein, C.H., Ivanciuc O. and **Braun, W.** Bioinformatics Approaches to Classifying Allergens and Predicting Cross-reactivity. Immunol. Allergy Clin. North Am., (in press) 2007.

Chen, D, Menche, G, Power, T.D., Peterson, J.W. and **Schein, C.H.** Accounting for Ligand-bound Metal Ions in Docking Small Molecules on Adenylyl Cyclase Toxins. Proteins, (in press) 2007.

McGehee, T.J., Rangasetty, U.C., Atar, S., Barbagelata, A.N., Uretsky, B.F., **Birnbaum, Y..** Grade 3 ischemia on admission ECG and chest pain duration predict failure of ST segment resolution following primary percutaneous coronary intervention for acute myocardial infarction. Journal of Electrocardiology 2007; 40:26-33.

Malik, M., **Birnbaum, Y.,** Macleod, R.S., Shusterman, V.. Markers of impaired repolarization. Journal of Electrocardiology 2007: (1 Suppl):S54-57.

Birnbaum, Y., Wagner, G.. Pseudo-ST-elevation acute myocardial infarction. Journal of Electrocardiology 2007; (1 Suppl): S45-46.

Merla, R., Adams, G., **Birnbaum, Y.,** Uretsky, B., Wagner, G., Barbagelata, A.. Wireless electrocardiogram in early diagnosis and triaging of ST-elevation myocardial infarction: The TIME Study. Journal of Electrocardiology 2007; (1 Suppl):S39-41.

Birnbaum, Y. The consideration of electrocardiographic ischemia grading to predict ST resolution with reperfusion therapy for ST elevation acute myocardial infarction. Journal of Electrocardiology. 2007; 40(1 Suppl):S32-33.

Continued on next page

Publications & Grant Awards (cont.)

Birnbaum, Y. The burden of non-ischemic ST elevation. Journal of Electrocardiology 2007;40:6-9.

Rosanio, S., Schwarz, E.R., Vitarelli, A., Zarraga, I.G., Kunapuli, S., Ware, D.L., **Birnbaum, Y**., Tuero, E., Uretsky, B.F.. Sudden death prophylaxis in heart failure. International Journal of Cardiology 2007 Jan 4; [in press]

Birnbaum, Y., Ye, Y., Lin, Y., Freeberg, S.Y, Huang, M-H., Perez-Polo, J.R., Uretsky, B.F.. Aspirin augments 15-epi-lipoxin A4 production by lipopolysaccharide, but blocks the pioglitazone and atorvastatin induction of 15-epi-lipoxin A4 in the rat heart. Prostaglandins and Other Lipid Mediators 2007;83:89-98.

Birnbaum, Y., Lin, Y., Ye, Y., Martinez, J.D., Huang, M-H., Lui, C.W., Perez-Polo, J.R., Uretsky, B.F.. Aspirin before reperfusion blunts the infarct size limiting effect of atorvastatin. American Journal of Physiology; Heart and Circulatory Physiology 2007, [in press]

GRANTS

W. Bujalowski, 2 R01 GM058565-09, Functional Dynamics of Mammalian and Viral DNA Repair Polymerases, four years of support with a total award of $1.1 million dollars.

V. J. Hilser received a grant from the Mitchell Center for Neurodegenerative Diseases for the development of peptide inhibitors and stimulators of amyloid formation in the prion protein. Award: 2 years at $70K total

V. J. Hilser received a WIRED grant from the Western Regional Center of Excellence for the development of viral inhibitors targeted to the S protein of the SARS coronavirus. Award: 2 years at $295K total.

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

Administrator's Notes

The three new faculty members who joined BMB in January are setting up their laboratories in the newly-completed suite on the 6th floor of the Basic Science Building. Yvette Boyd is providing administrative assistance to Dr. Junji Iwahara, and Rose Byrdlon-Griggs is assisting Drs. Kay Choi and Marc Morais.

We certainly miss Diane Strain, who has had some setbacks in recovering from her shoulder injury in December. The Administrative Staff along with the Papaconstantinou and Carney groups are continuing to stay in touch with Diane and her family and are sending her news about BMB activity. We appreciate very much all the effort that Terry Campbell, Yvette Boyd, and Rose Byrdlon-Griggs are giving to covering the critical elements of Diane's work while they carry out their regular responsibilities.

Featured Abstract by BMB Faculty

Chen, D, Menche, G, Power, T.D., Peterson, J.W. and ***Schein, C.H.*** *Accounting for Ligand-bound Metal Ions in Docking Small Molecules on Adenylyl Cyclase Toxins.* ***Proteins****, (in press) 2007.*

The adenylyl cyclase toxins produced by bacteria (such as the edema factor (EF) of *Bacillus anthracis* and the CyaA of *Bordetella pertussis*) are important virulence factors in anthrax and whooping cough. Co-crystal structures of these proteins differ in the number and positioning of metal ions in the active site. Metal ions bound only to the ligands in the crystal structures are not included during the docking. To determine what effect these "missing" metals would have on docking results, the AutoDock, LigandFit/Cerius2, and FlexX programs were compared for their ability to correctly place substrate analogues and inhibitors into the active sites of the crystal structures of EF, CyaA, and mammalian adenylate cyclase. Protonating the phosphates of substrate analogues improved the accuracy of docking into the active site of CyaA, where the grid did not account for one of the Mg2+ ions in the crystal structure. The AutoDock ranking (based on docking energies) of a test group of compounds was relatively unaffected by protonation of carboxyl groups. However, the ranking by FlexX-ChemScore varied significantly, especially for docking to CyaA, suggesting that alternative protonation states should be tested when screening compound libraries with this program. When the charges on the bound metal were set correctly, AutoDock was the most reliable program of the three tested with respect to positioning substrate analogues and ranking compounds according to their experimentally determined ability to inhibit EF.

BMB congratulates the faculty and staff members to be awarded Service Pins at the Employee Service Day ceremony on February 15. A combined total of 155 Years of Service to UT components!

5 Years of Service
Jianhang Jia, Ph.D., Assistant Professor, Biochemistry (BMB and the Sealy Center for Cancer Cell Biology)
Richard Kanost, Research Associate (Molecular Genomics Core Service)
Aditi Das, Ph.D., Post-doctoral Fellow, (Mitra Group)
Julieann Lee, Research Associate (Perez-Polo Group)
Shriram Srivastava, Research Associate (N. Ansari Group)
Debora Botting, Special Program Coordinator, Graduate Programs

10 Years of Service
Catherine Schein, Ph.D., Associate Professor of Biochemistry
John (Steve) Hathorn, Lab Services Supervisor

15 Years of Service
Sankar Mitra, Ph.D., Professor, Biochemistry (BMB and the Sealy Center for Molecular Medicine)

Deborah Prusack, Molecular Biologist, (Molecular Genomics Core Service)
Maribel Acosta, Molecular Biologist, (Molecular Genomics Core Service)
Shirley Broz, Senior Administrative Secretary (Lee Group and the Sealy Center for Molecular Medicine)

20 Years of Service
Angelina Johnson, Senior Administrative Secretary (Hilser Group and the Sealy Center for Structural Biology)

25 Years of Service
Mamie Barnard, formerly Senior Administrative Secretary (Molecular Genomics Core Service)

The University of Texas Medical Branch
Department of Biochemistry and Molecular Biology
presents

The Mary Huling Edens Memorial Lecture Series Spring 2007

February 8, 2007 4:00 pm Basic Science Auditorium	Harold K. Kimelberg, Ph.D. Senior Scientist, Ordway Research Institute Director, Nerve Cell Rescue Laboratory Adjunct Professor, Center for Neuroscience & Neuropharmacology Albany Medical College Department of Biology State University of New York at Albany	"Astroglial cell swelling in acute CNS pathologies (stroke and traumatic brain injury"
February 20, 2007 5:00 pm Levin Hall	Joseph G. Verbalis, M.D. Professor of Medicine and Physiology Interim Chair, Department of Medicine Georgetown University Medical Center, Physician in Chief Georgetown University Hospital Washington, DC	"Cell Volume Regulation in vivo: Adaptation or Maladaptation?"
March 22, 2007 4:00 pm Basic Science Auditorium	Clive Baumgarten Virginia Commonwealth University	TBA
April 12, 2007 4:00 pm Basic Science Auditorium	Mortimer M. Civan, Ph.D. Professor of Physiology Department of Physiology University of Pennsylvania	"Chloride channels and the aqueous humor. On the one side and the other"

All interested persons are welcome to attend

The Mary Huling Edens Lectureship in Medical Genetics was established in 1975 by Dr. Lee Edward Edens, UTMB School of Medicine Class of 1921, in memory of his wife.

Faculty on the Road

Dr Yogesh Awasthi attended a Xenobiotic and Nutrient Disposition and Action (XNDA) Study Section in Bethesda, MD; February 6-8, 2007.

Dr. Tapas Hazra traveled to Ventura, CA on February 4-9 to attend the Gordon Research Conference on Mammalian DNA repair.

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.

New Employees

Kyung (Kay) Choi, Ph.D., Assistant Professor, Biochemistry

Marc Morais, Ph.D. Assistant Professor, Biochemistry

Zhezong Luo, Research Associate II working with Dr. Mitra.

Alex Torres, College Intern working with

Dr. Naseem Ansari.

Jade Truong, College Intern working with

Dr. Naseem Ansari.

You Count Survey Deadline is Friday, February 23

All BMB faculty, staff, and students are encouraged to respond to the You Count Survey. The UTMB Administration values the information obtained through the survey, which provides the opportunity to give input through a confidential process that does not reveal individual respondents' identities.

Questions? Please call Human Resources--Organizational Effectiveness, Training & Recognition at (409) 747-6700, send an email to youcount.hr@utmb.edu, or visit the You Count web site at www.utmb.edu/youcount.

New - ONLINE Version
Research Coordinator's Corner
www.bmb.utmb.edu/department/RCC/

Dr. Konkel's Column will not be published this month.

Previously published columns are available through the link above.