



Life Sciences Division

E-Newsletter January 31, 2008

The LBNL Life Sciences Newsletter is reorganized starting with this issue in order to highlight DOE scientific focus area research within the Life Sciences Division.

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DOE scientific focus area notes

Low Dose Radiation Research

Investigator's Workshop and Int. Workshop in Systems Radiation Biology

The Low Dose Investigator's Workshop held in Washington, D.C. January 21-23, 2008 featured keynote talks by LBNL investigators, **Terumi Kowhi-Shigamatsu** and **Mina Bissell**. Dr. Kowhi-Shigamatsu discussed her novel observations about how chromatin composition and organization affect how the genome is used. She discussed how SATB1, which is a nuclear protein whose purpose is to integrate co-regulated genes, establishes local histone modification code. She suggested that DNA damage induced histone phosphorylations are co-ordinately regulated with SATB1 networks, which provides a new level of complexity relating nuclear architecture to DNA repair capacity. Dr. Bissell presented her recent research expanding on her long standing program defining how cells integrate and respond to critical signals received from the microenvironment. Exciting new studies show how this integration fails in cancer, and the ability to revert malignant behaviors by intervening in the signaling at the cell surface. The meeting was summarized by **Mary Helen Barcellos-Hoff** in her role as program chief scientist.

The 2nd International Workshop in Systems Radiation Biology sponsored by DOE Low Dose program was held immediately after the investigators workshop. LBNL researchers were actively involved in this collaborative effort with the European Community programs in radiation research and NASA to initiate systems biology approaches to low dose radiation biology problems. **Mary Helen Barcellos-Hoff** was chair and LBNL investigators, Adam Arkin, **Sylvain Costes**, **Bahram Parvin** and **Andrew Wyrobek** presented their research to an audience of about 60 researchers from around the world. Given the current research goal to determine the consequences of high and low radiation exposures, broadening the scope of radiation studies to include systems biology concepts should benefit risk modeling of radiation carcinogenesis. As a result of last year's workshop, a new publication entitled "Cancer as an emergent phenomenon in systems radiation biology" by **Barcellos-Hoff** has appeared in *Radiat Environ Biophys* 2008 Feb;47(1):33-8. Epub 2007 Nov 20 (PMID: 18026977).

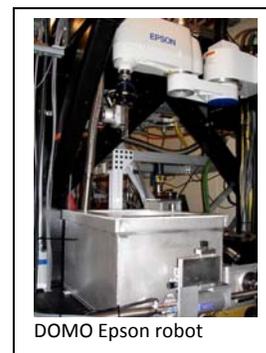
Mary Helen Barcellos-Hoff, 1/08

GTL-Genomics

SIBYLS Beamline Goes Robotic

Both the Small Angle X-ray Scattering (SAXS) and Macromolecular Crystallography (MX) endstations at the SIBYLS beamline have recently installed robotic systems for the automation of sample loading. SIBYLS beamline users routinely load their SAXS samples into the sample cell using a Hamilton Microlab 4000 liquid handling robot. What used to entail an elaborate and time consuming procedure of accessing the experimental hutch, washing the sample cell, and changing samples has now been simplified to a few clicks of the mouse. The time and effort saved is priceless. Additionally, the MX endstation has installed a heavily modified Epson robotic arm that can pick frozen protein crystals from liquid nitrogen and place them in position to collect X-ray diffraction data. Referred to as DOMO (Dynamic Offsite MX Operator), the new Epson robot arm in combination with improved beamline operating software, has allowed researchers in San Diego to collect data remotely from the comfort of

their home labs; thus saving them the time to travel to LBNL. These two technical advances hold great promise to improve the efficiency and productivity of the SIBYLS beamline users. By automating both MX and SAXS a unique facility is provided to efficiently integrate solution scattering for shape, conformation, and assembly along with crystallography for high resolution. MX coupled to SAXS helps to address the challenges of multiple conformations, large assemblies, and functionally-important flexibility that are important to DOE GTL and bioenergy missions as well as NIH missions in cancer biology relevant to novel therapeutic strategies. Further information can be found on the SIBYLS website at <http://bl1231.als.lbl.gov>.



Publications

Interactions and Dynamics of the Shine–Dalgarno Helix in the 70S Ribosome. Andrei Korostelev, Sergei Trakhanov, Haruichi Asahara, Martin Laurberg, Laura Lancaster, and Harry F. Noller. Center for Molecular Biology of RNA and Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, CA 95060. Proceedings of the National Academy of Sciences 104: 16840-16843.

The crystal structure of an initiation-like 70S ribosome complex containing an 8-bp Shine–Dalgarno (SD) helix was determined at 3.8-Å resolution. Translation–libration–screwanalysis showed that the inherent anisotropic motions of the SD helix were biased along its helical axis, suggesting that during the first step of translocation, the SD helix moves along its helical screw axis. Contacts between the SD helix and the ribosome were primarily through interactions with helices 23a, 26, and 28 of 16S rRNA. Contact with the neck (helix 28) of the 30S subunit near its hinge point suggests that formation of the SD helix could affect positioning of the head of the 30S subunit for optimal interaction with initiator tRNA. The bulged U723 in helix 23a interacts with the minor groove of the SD helix at the C1539-G-10 base pair, explaining its selective conservation in bacteria and archaea.

Work in the following Nature paper of Physical Biosciences scientist John Kuriyan was supported heavily by the SIBYLS beamline (12.3.1):

Inhibition of the EGF receptor by binding of MIG6 to an activating kinase domain interface. Xuewu Zhang^{1,4}, Kerry A. Pickin², Ron Bose^{2,4}, Natalia Jura¹, Philip A. Cole² & John Kuriyan. Nature 450, 741-744 (29 November 2007).

Members of the epidermal growth factor receptor family (EGFR/ERBB1, ERBB2/HER2, ERBB3/HER3 and ERBB4/HER4) are key targets for inhibition in cancer therapy¹. Critical for activation is the formation of an asymmetric dimer by the intracellular kinase domains, in which the carboxy-terminal lobe (C lobe) of one kinase domain induces an active conformation in the other². The cytoplasmic protein MIG6 (mitogen-induced gene 6; also known as ERFF11) interacts with and inhibits the kinase domains of EGFR and ERBB2 (refs 3–5). Crystal structures of complexes between the EGFR kinase domain and a fragment of MIG6 show that a 25-residue epitope (segment 1) from MIG6 binds to the distal surface of the C lobe of the kinase domain. Biochemical and cell-based analyses confirm that this interaction contributes to EGFR inhibition by blocking the formation of the activating dimer interface. A longer MIG6 peptide that is extended C terminal to segment 1 has increased potency as an inhibitor of the activated EGFR kinase domain, while retaining a critical dependence on segment 1. We show that signaling by EGFR molecules that contain constitutively active kinase domains still

requires formation of the asymmetric dimer, underscoring the importance of dimer interface blockage in MIG6-mediated inhibition.

Scott Classen/John Tainer, 1/08

PCAP's Single Particle EM Effort

The Genomics GTL Protein Complex Analysis Project (PCAP) has two major goals: 1. to develop an integrated set of high throughput pipelines to identify and characterize multi-protein complexes in a microbe more swiftly and comprehensively than currently possible and 2. to use these pipelines to elucidate and model the protein interaction networks regulating stress responses in *Desulfovibrio vulgaris* with the aim of understanding how this and similar microbes can be used in bioremediation of metal and radionuclides found in U.S. Department of Energy (DOE) contaminated sites. The Single-particle EM effort within PCAP has focused during the first two years on large soluble-protein complexes with Mr in the range 400 k to over 1000 k. These complexes have been found to differ considerably in terms of how well they hold up during EM sample preparation, and not all are stable even under the currently used conditions of cryo-EM sample preparation. Roughly half of the 15 complexes studied have been stable enough to produce high-quality 3-D reconstructions, however, and class-average projection-images have been obtained for most of the others. This preliminary phase of characterization has shown surprising differences in the quaternary structures of complexes isolated from *DvH* and those that are already known for homologous proteins from other microbes. These differences occur frequently enough to make it clear that structures determined for other microorganisms are inadequate for use as templates for modeling the biochemical networks within a given microbe of interest. By extension it is clear that the same type of EM structure determinations could be essential to characterize any changes in the multi-protein complexes that exist under different physiological conditions.

Mark Biggin, 1/08

Nuclear Medicine

Recent Publications

Cherepy NJ, Kuntz JD, Tillotson TM, Speaks DT, Payne SA, Chai BHT, Porter-Chapman Y and **Derenzo SE**. Cerium-doped single crystal and transparent ceramic lutetium aluminum garnet scintillators. Nuclear Instruments & Methods in Physics Research Section A-Accelerators Spectrometers Detectors and Associated Equipment 2007; 579:38-41.

For rapid, unambiguous isotope identification, scintillator detectors providing high-resolution gamma ray spectra are required. We have fabricated Lutetium Aluminum Garnet (LuAG) using transparent ceramic processing, and report a 2-mm thick ceramic exhibiting 75% transmission and light yield comparable to single-crystal LuAG:Ce. The LuAG:Ce luminescence peaks at 550nm, providing an excellent match for Silicon Photodiode readout. LuAG is dense (6.67 g/cm³) and impervious to water, exhibits good proportionality and a fast decay (similar to 40ns), and we measure light yields in excess of 20,000 photons/MeV.

Porter-Chapman Y, Bourret-Courchesne E and **Derenzo SE**. Bi³⁺ luminescence in ABiO(2)Cl (A = sr, ba) and BaBiO2Br. Journal of Luminescence 2008; 128:87-91.

Trivalent bismuth luminescence is reported in three Sillen bismuth oxyhalide phases, SrBiO2Cl, BaBiO2Cl, and BaBiO2Br. These compounds exhibit Bi 6s6p -> 6s(2) emission under UV and X-ray radiations. At room temperature, BaBiO2Cl shows the most intense light emission, with spectral and decay properties similar to those found in Bi4Ge3O12 (BGO). At low temperatures, each phase show an increase in the photoluminescence intensities and a narrowing of the emission peaks. In contrast to the

temperature dependence of BGO, X-ray excited luminescence intensities of all three phases remain relatively constant throughout the temperature range 10-295 K, though much lower than BGO at low temperatures. This result indicates that the Sillen phases undergo less thermal quenching than BGO. The low temperature and room temperature radio-luminescence decay times were determined from Pulsed X-ray measurements. At room temperature, SrBiO₂Cl exhibits faster decays than BGO, while BaBiO₂Cl and BaBiO₂Br have decay times similar to BGO.

Stephen Derenzo, 1/08

Scientific news

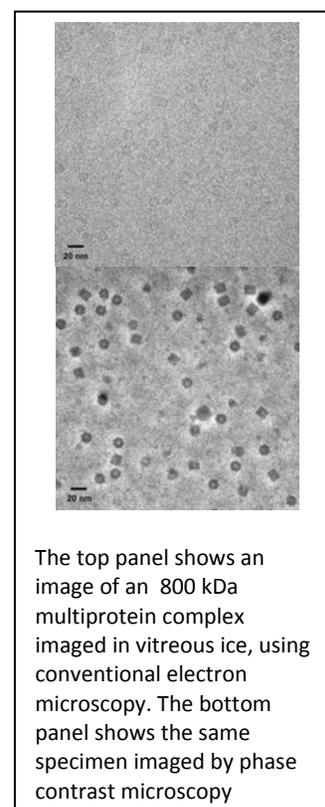
Division Cryo-electron Microscopy of Biological Nanostructures

The January 2008 issue of *Physics Today* carries two Feature Articles on biophysical topics. The first, by William Gelbart and Charles Knobler at UCLA, is titled "The Physics of Phages", and the second, by **Robert Glaeser**, a member of both the Life Sciences Division and the Physical Biosciences Division, is titled "Cryo-electron Microscopy of Biological Nanostructures". The cover of this issue features an image of P22 phage obtained by the laboratory of Wah Chiu, a former graduate student in the Life Sciences Division and now a professor at Baylor College of Medicine in Houston. LBNL was one of the birthplaces of cryo-EM in the early 1970's. In work supported by BER within DOE, Kenneth Taylor, then a graduate student here and now a professor at Florida State Univ., was the first to demonstrate that the native structure of protein molecules could be preserved in the vacuum of the electron microscope by using thin, "frozen hydrated" specimens. It was not until 1990, in cryo-EM work on the membrane protein bacteriorhodopsin, which was led by Richard Henderson in Cambridge, England, that the technology had advanced to the stage that three-dimensional reconstructions could be interpreted in atomic detail. Electron micrographs recorded by **Kenneth Downing** in the Life Sciences Division played a crucial role in advancing the work to the resolution required to build such a model of the structure. In 1998, **Eva Nogales**, then a postdoctoral fellow in Downing's lab and now a professor and HHMI Investigator on the Berkeley Campus as well as a member of both the Life Sciences and Physical Biosciences Divisions, together with Downing and Sharon Wolf, used cryo-EM to solve the structure of tubulin, which - though highly sought after because of its importance in the mitotic spindle and other cytoskeletal structures - had resisted efforts to be studied by X-ray crystallography.

[Full Story]

<http://ptonline.aip.org/dbt/dbt.jsp?KEY=PHTOAD&Volume=LASTVOL&Issue=LASTISS#MAJOR1>

Robert Glaeser, 1/08



Beebe Symposium – 60th Anniversary for the ABCC/RERF



For the past 60 years, the National Academies have studied the health effects of radiation exposure in survivors of the atomic bombs dropped on Hiroshima and Nagasaki, Japan. To commemorate the many contributions of former and current research employees and bomb survivors, the National Academy of Sciences hosted a symposium, "Sixty Years of ABCC/RERF: Major Contributions and Future Studies" in Washington D.C. on Dec. 12. Berkeley Lab Associate Laboratory Director **Joe Gray** gave the keynote speech in the section on "The Future", focusing on exploiting new technologies in radiation research.

[Full story] <http://www.prnewswire.com/cgi-bin/stories.pl?ACCT=104&STORY=/www/story/12-05-2007/0004717386&EDATE>
[Symposium] <http://dels.nas.edu/nrsb/meetings.shtml>
Today at Berkeley Lab, 12/7/07

Thinking Outside Cell Key to Cancer Research

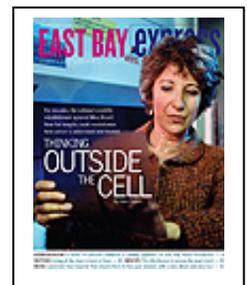
Why do we get cancer? For years, conventional wisdom held that cancer begins solely with a DNA mutation that causes cells to run amok and reproduce uncontrollably. Berkeley Lab life scientist **Mina Bissell** believes a crucial part of cancer formation is not just what goes wrong inside the cell, but what goes wrong in the way it interacts with its extracellular matrix, the 3-D architecture that surrounds and supports the cell. If Bissell is right, her insight will revolutionize not only how cancer is understood and treated, but perhaps what it means to have the disease.

[Full story]

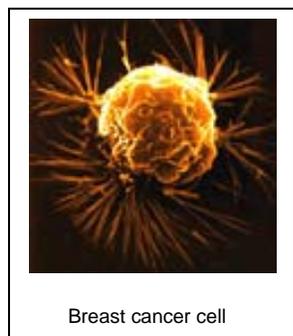
http://www.eastbayexpress.com/news/thinking_outside_the_cell/Content?oid=600256

[More] http://www.eastbayexpress.com/news/the_structure_is_the_message/Content?oid=600259

Today at Berkeley Lab, 12/13/07



How Cell is Affected by its 'Neighborhood'



Breast cancer cell

Mina Bissell, Life Sciences Division Distinguished Scientist at Berkeley Lab, is renowned for her discovery that the cell's microenvironment determines how genes function in cancer and other diseases. Bissell's breakthrough inspired the recent work referenced in a *Los Angeles Times* article, quoting **Mark LaBarge** in Bissell's lab. Much of the research will be forthcoming soon, LaBarge says, but the article's inspiration was a *Cancer Cell* paper by Bissell and him on the how the microenvironment regulates tumor stem cells; it concludes, "Understanding which cues stimulate a stem cell to get activated may lead to prophylactic approaches for therapy and possible prevention."

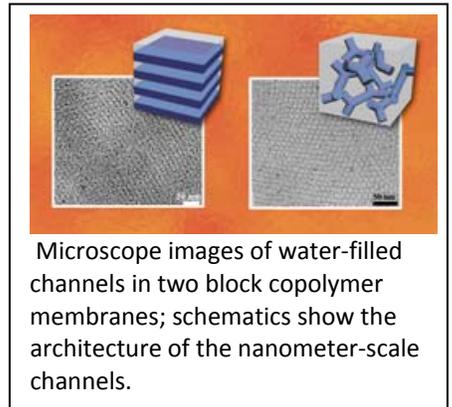
Read the paper here: http://www.lbl.gov/today/2007/Dec/18-Tue/bissel-labarge-Cancer-Cell_2005.pdf

Today at Berkeley Lab, 12/18/07

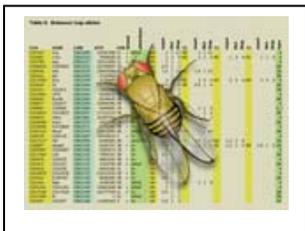
Hot Wet Polymers

Copolymer membranes that get wetter as the surrounding air gets hotter have been developed by Nitash Balsara of the Materials Sciences Division, leading a team that includes fellow MSD members Moon Jeong Park, Enrique Gomez, and Andrew Minor, plus **Ken Downing** of Life Sciences, Adam Weber of the Environmental Energy Technologies Division, and colleagues from NIST and Argonne National Laboratory. Polymer electrolyte fuel cells show promise as a nonpolluting power source for cars and other applications, but when they get hot they lose water and suffer a decrease in proton conductivity. The unlikely new materials could change that. Made from nanoscale layers of two kinds of polymer, one that repels water and another that attracts it, the copolymers are riddled with hydrophilic channels. When channels measure less than five nanometers across, water uptake increases as the temperature rises, and proton conductivity increases at the same time — ideal for fuel cells. The oddball polymers are described in the Oct. 26 online version of Nano Letters. More Porous Polymers: Another way nano-openings in polymers could help advance the energy frontier is by boosting the ability of fuel cells to store hydrogen, thus overcoming the problems associated with compressing and liquefying the gas. Frantisek (Frank) Svec of MSD, working with MSD's Jean Fréchet and Jonathan Germain of UC Berkeley, has come up with a potential adsorbent for hydrogen made of “hypercrosslinked polyanilines with nanoporous structure and high surface area.” Polyanilines are rod-shaped conducting polymers, and these polyanilines have high affinity for hydrogen. Hypercrosslinking them creates a mesh-like structure that keeps the polymer chains apart, leaving a material that's riddled with nanometer-sized pores and has an enormous surface area for its diminutive bulk — up to eight times higher than previous porous polyanilines. More surface grabs more hydrogen, potentially providing a solution to the problem of hydrogen storage in fuel tanks. The researchers published their work online October 18 in the Journal of Materials.

Berkeley Lab View, 12/14/07



Not Your Great-Grandfather's Fruit Fly



Drosophila melanogaster has been used to study genetics for over a century. *Drosophila melanogaster* led genetic research for over a century, the model organism that pioneered many of the basic principles governing animal development and population biology and provided the first-sequenced whole genome of a complex animal. But *Drosophila* is not the only species of fruit fly, and now members of Harvard's FlyBase and the Berkeley *Drosophila* Genome Project, including the Life Sciences Division's **Joseph Carlson** and

Susan Celniker, have sequenced, assembled, and annotated the genomes of 12 different *Drosophila* species to discover new functional elements in the genome. In addition to shedding new light on the evolution of genes and chromosomes and how they relate to speciation and adaptation in *Drosophila*, a major goal was to develop general methods for discovering functional elements and their implications for vertebrate studies. The major research appeared in the November 8 issue of *Nature*.

Berkeley Lab View, 12/14/07

Meeting Looks at Future of Biosciences Research

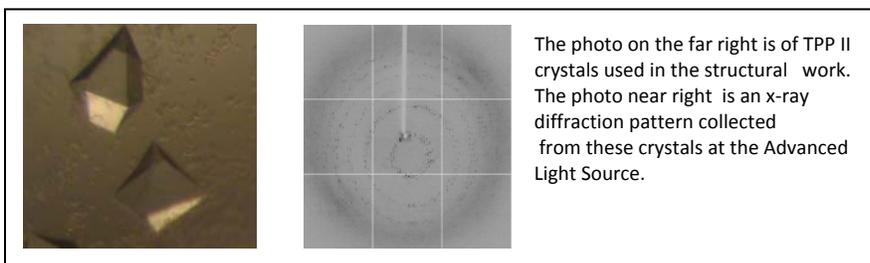
Seeking to further the innovation in biosciences research, a symposium on “Accelerating Innovation in 21st Century Biosciences: Identifying the Measurement Standards and Technological Challenges” will be held Oct. 20-24, 2008 in Gaithersburg, MD. The gathering will be “focused on identifying and prioritizing measurement, standards, and technology needs that represent barriers to innovation, and impediments to achieving maximal societal and economic benefits of new discoveries in the biosciences.” Berkeley Lab life scientist **Tamas Torok** is a member of the steering/program committee for the symposium. Go here for more information: <http://www.cstl.nist.gov/Biosciences.html>

Today at Berkeley Lab, 1/22/08

Awards

NIH General Medicine Institute Award for Bing Jap

Bing Jap’s group has recently been awarded a grant by the General Medicine Institute of the NIH to conduct the structural studies necessary to reveal the molecular mechanism of Tripeptidyl peptidase II (TPP II) mediated peptidolysis, complex assembly, and assembly-dependent activation. Much of the work will focus on the structure determination of this protease at atomic resolution and utilize the resources of the Advanced Light Source at LBNL. This protein complex is a component of the ubiquitin-proteasome cascade that is the major system for proteolysis of cytosolic proteins in eukaryotic cells. Most of the proteasome-generated oligopeptides require further processing by peptidases. TPP II, a 6 MDa



subtilisin-like serine peptidase complex displaying both exo- and endo-peptidolytic activities, cleaves proteasome products to produce MHC class I antigenic peptides and short peptides that can be used as substrates by other exopeptidases. It is the primary peptidase for the processing of proteasome-generated peptides 14-17 amino acids in length. Interestingly, it has been found to be able to work independently of the proteasome as observed in the case where TPP II mediated endo-peptidolytic activity generates the human immunodeficiency virus (HIV) epitope, Nef. TPP II also appears to be a peptidase critical for cell survival in cases where proteasome activity is sub-optimal, such as has been observed in apoptosis-resistant tumor cells. A membrane-bound isoform of TPP II participates in the proteolytic processing of cholecystokinin (CCK) family members involved in physiological processes such as memory and a diverse range of psychiatric disorders including psychosis and eating disorders.

By Bing Jap, 1/08

Tech Transfer Awards Go to Inventors in 7 Projects

Ten Berkeley Lab scientists were honored this week for their significant contributions to the technology transfer process through inventing, working with Tech Transfer Office staff, and interacting with companies. In a brief ceremony Tuesday, Lab Director **Steve Chu** presented 2007 Technology Transfer Awards to the following researchers whose technologies in seven projects were licensed this year:

Tamas Torok, Tom Budinger and **Jonathan Maltz** of Life Sciences; Paul Alivisatos, Steven Hughes, Richard Robinson, and Bryce Sadtler of Materials Sciences; Jay Groves of Physical Biosciences; Ted Chang of Environmental Energy Technologies; and Nitash Balsara of Materials Sciences and EETD. Read more about the technology and to view a full-size image of the recipients here:

<http://www.lbl.gov/today/2007/Dec/13-Thu/tech-transfer-jump.html>

Today at Berkeley Lab, 12/13/07

Breast Cancer Expert Gets International Award

The French National Institute for Health and Medical Research (INSERM), a public scientific and technological organization, has given Berkeley Lab life scientist **Mina Bissell** its "Annual International Award" for an outstanding individual in science, medicine or public health that has motivated and reinforced international exchange and collaboration in terms of innovation and improvement of excellence and the transfer of fundamental research to clinical research and public health. Bissell was recognized for her "three decades of creativity, focus and tenacity to uncover the essential role of cell and matrix topology in carcinogenesis with recent elucidation of the mechanisms by which tissue geometry can control branching morphogenesis by defining the local microenvironment. Your work has already significantly benefited sciences and medicine and grows in ever increasing directions."

Berkeley Lab View, 12/14/07 (also in Today at Berkeley Lab, 12/11/07)

Gray Named Winner of Komen Research Award

The Susan G. Komen Breast Cancer Foundation recently announced the 2007 winners of its Brinker Award for Scientific Distinction. The award recognizes leading scientists for significant work that advances basic research concepts or clinical application in the fields of breast cancer. This year's recipients are Berkeley Lab Associate Laboratory Director **Joe Gray** and Leslie Bernstein of the University of Southern California, Los Angeles. Komen Foundation press release:

http://cms.komen.org/komen/NewsEvents/071213_2007_Brinker_Award

Today at Berkeley Lab, 12/13/07

Lab's Bissell Namesake of New Research Award

At the recent Symposium on Frontiers in Cell Migration in Cancer, organized by the Institute of Molecular Pathology and Immunology at Portugal's University of Porto, an award named after Berkeley Lab Senior Scientist **Mina Bissell** was unveiled. Bissell then presented the first-time award to Leonor Beza, president of the Champalimaud Foundation. The award will be given every two years to a scientist who "like Bissell, has a devoted successful, lifetime research career that has transformed our perception of a topic.

Today at Berkeley Lab, 1/28/08

Luncheon Honors Runaround Veteran

The Lab's Employee Activities Association has honored **Steve Derenzo**, senior scientist in the Life Sciences Division, for his 27 years of coordinating the Lab's annual Runaround race. An informal luncheon recognizing his service was held on December 18, 2007.

Today at Berkeley Lab, 12/18/07

Recent publications (selected)

Andarawewa KL, Paupert J, Pal A, **Barcellos-Hoff MH**. New rationales for using TGFbeta inhibitors in radiotherapy. Int J Radiat Biol. 2007 Nov;83(11):803-11. PMID: 18058368

The first reports that ionizing radiation (IR) induces rapid and persistent activation of transforming growth factor beta1 (TGFbeta) were nearly two decades ago. Subsequent studies have shown that TGFbeta is a major mediator of cellular and tissue responses to IR and have revealed novel facets of its complex biology. Results: We and others have recently shown that inhibition of production or signaling of TGFbeta in epithelial cells modulates radiosensitivity and impedes activation of the DNA damage response program. The primary transducer of cellular response to DNA damage caused by ionizing radiation is the nuclear protein kinase ataxia telangiectasia mutated, whose activity is severely compromised when TGFbeta is inhibited. Thus, in conjunction, with its well-recognized contribution to normal tissue fibrosis, the role of TGFbeta in the genotoxic stress program provides a previously unsuspected avenue to modulate radiotherapy. Conclusions: We hypothesize that identification of the circumstances and tumors in which TGFbeta manipulation enhances tumor cell radiosensitivity, while protecting normal tissues, could significantly increase therapeutic index.

Rizki A, Mott JD, Bissell MJ. Polo-like kinase 1 is involved in invasion through extracellular matrix. Cancer Res. 2007 Dec 1;67(23):11106-10 PMID: 18056432

Polo-like kinase 1 (PLK1) has important functions in maintaining genome stability via its role in mitosis. Because PLK1 is up-regulated in many invasive carcinomas, we asked whether it may also play a role in acquisition of invasiveness, a crucial step in transition to malignancy. In a model of metaplastic basal-like breast carcinoma progression, we found that PLK1 expression is necessary but not sufficient to induce invasiveness through laminin-rich extracellular matrix. PLK1 mediates invasion via vimentin and beta1 integrin, both of which are necessary. We observed that PLK1 phosphorylates vimentin on Ser82, which in turn regulates cell surface levels of beta1 integrin. We found PLK1 to be also highly expressed in preinvasive in situ carcinomas of the breast. These results support a role for the involvement of PLK1 in the invasion process and point to this pathway as a potential therapeutic target for preinvasive and invasive breast carcinoma treatment.

Hudson SG, Garrett MJ, Carlson JW, Micklem G, **Celniker SE**, Goldstein ES, Newfeld SJ. Phylogenetic and genomewide analyses suggest a functional relationship between kayak, the Drosophila fos homolog, and fig, a predicted protein phosphatase 2c nested within a kayak intron. Genetics. 2007 Nov;177(3):1349-61. PMID: 18039871

A gene located within the intron of a larger gene is an uncommon arrangement in any species. Few of these nested gene arrangements have been explored from an evolutionary perspective. Here we report a phylogenetic analysis of kayak (kay) and fos intron gene (fig), a divergently transcribed gene located in a kayak intron, utilizing 12 *Drosophila* species. The evolutionary relationship between these genes is of interest because kayak is the homolog of the proto-oncogene c-fos whose function is modulated by serine/threonine phosphorylation and fig is a predicted PP2C phosphatase specific for serine/threonine residues. We found that, despite an extraordinary level of diversification in the intron-exon structure of kayak (11 inversions and six independent exon losses), the nested arrangement of kayak and fig is conserved in all species. A genomewide analysis of protein-coding nested gene pairs revealed that approximately 20% of nested pairs in *D. melanogaster* are also nested in *D. pseudoobscura* and *D. virilis*. A phylogenetic examination of fig revealed that there are three subfamilies of PP2C phosphatases in all 12 species of *Drosophila*. Overall, our phylogenetic and genomewide analyses suggest that the nested arrangement of kayak and fig may be due to a functional relationship between them.

Parra MK, Tan JS, Mohandas N, **Conboy JG**. Intraspllicing coordinates alternative first exons with alternative splicing in the protein 4.1R gene. EMBO J. 2008 Jan 9;27(1):122-31. Epub 2007 Dec 13. PMID: 18079699

In the protein 4.1R gene, alternative first exons splice differentially to alternative 3' splice sites far downstream in exon 2'/2 (E2'/2). We describe a novel intrasplicing mechanism by which exon 1A (E1A) splices exclusively to the distal E2'/2 acceptor via two nested splicing reactions regulated by novel properties of exon 1B (E1B). E1B behaves as an exon in the first step, using its consensus 5' donor to splice to the proximal E2'/2 acceptor. A long region of downstream intron is excised, juxtaposing E1B with E2'/2 to generate a new composite acceptor containing the E1B branchpoint/pyrimidine tract and E2 distal 3' AG-dinucleotide. Next, the upstream E1A splices over E1B to this distal acceptor, excising the remaining intron plus E1B and E2' to form mature E1A/E2 product. We mapped branchpoints for both intrasplicing reactions and demonstrated that mutation of the E1B 5' splice site or branchpoint abrogates intrasplicing. In the 4.1R gene, intrasplicing ultimately determines N-terminal protein structure and function. More generally, intrasplicing represents a new mechanism by which alternative promoters can be coordinated with downstream alternative splicing.

Krauss SW, Spence JR, Bahmanyar S, Barth AI, Go MM, Czerwinski D, Meyer AJ. Downregulation of protein 4.1R, a mature centriole protein, disrupts centrosomes, alters cell cycle progression, and perturbs mitotic spindles and anaphase. Mol Cell Biol. 2008 Jan 22 [Epub ahead of print] PMID: 18212055

Centrosomes nucleate and organize interphase microtubules and are instrumental in mitotic bipolar spindle assembly, ensuring orderly cell cycle progression with accurate chromosome segregation. We report that the multifunctional structural protein 4.1R localizes at centrosomes to distal/subdistal regions of mature centrioles in a cell-cycle dependent pattern. Significantly, 4.1R-specific depletion mediated by RNA-interference perturbs subdistal appendage proteins ninein and ODF2/cenexin at mature centrosomes and concomitantly reduces interphase microtubule anchoring and organization. 4.1R-depletion causes G1 accumulation in p53-proficient cells, similar to depletion of many other proteins that compromise centrosome

integrity. In p53-deficient cells, 4.1R-depletion delays S-phase but aberrant ninein distribution is not dependent on the S-phase delay. In 4.1R-depleted mitotic cells, efficient centrosome separation is reduced resulting in monopolar spindle formation. Multipolar spindles and bipolar spindles with misaligned chromatin are also induced by 4.1R-depletion. Notably all types of defective spindles have mislocalized NuMA (Nuclear Mitotic Apparatus Protein), a 4.1R binding partner essential for spindle pole focusing. These disruptions contribute to lagging chromosomes and aberrant microtubule bridges during anaphase/telophase. Our data provide functional evidence that 4.1R makes crucial contributions to centrosome and mitotic spindle structural integrity which normally enable mitosis and anaphase to proceed with the coordinated precision required to avoid pathological events.

Groesser T, Chun E, **Rydberg B**. Relative biological effectiveness of high-energy iron ions for micronucleus formation at low doses. Radiat Res. 2007 Dec;168(6):675-82. PMID: 18088180

Dose-response curves for micronucleus (MN) formation were measured in Chinese hamster V79 and xrs6 (Ku80(-)) cells and in human mammary epithelial MCF10A cells in the dose range of 0.05-1 Gy. The Chinese hamster cells were exposed to 1 GeV/nucleon iron ions, 600 MeV/nucleon iron ions, and 300 MeV/nucleon iron ions (LETs of 151, 176 and 235 keV/microm, respectively) as well as with 320 kVp X rays as reference. Second-order polynomials were fitted to the induction curves, and the initial slopes (the alpha values) were used to calculate RBE. For the repair-proficient V79 cells, the RBE at these low doses increased with LET. The values obtained were 3.1 +/- 0.8 (LET = 151 keV/microm), 4.3 +/- 0.5 (LET = 176 keV/microm), and 5.7 +/- 0.6 (LET = 235 keV/microm), while the RBE was close to 1 for the repair-deficient xrs6 cells regardless of LET. For the MCF10A cells, the RBE was determined for 1 GeV/nucleon iron ions and was found to be 5.5 +/- 0.9, slightly higher than for V79 cells. To test the effect of shielding, the 1 GeV/nucleon iron-ion beam was intercepted by various thicknesses of high-density polyethylene plastic absorbers, which resulted in energy loss and fragmentation. It was found that the MN yield for V79 cells placed behind the absorbers decreased in proportion to the decrease in dose both before and after the iron-ion Bragg peak, indicating that RBE did not change significantly due to shielding except in the Bragg peak region. At the Bragg peak itself with an entrance dose of 0.5 Gy, where the LET is very high from stopping low-energy iron ions, the effectiveness for MN formation per unit dose was decreased compared to non-Bragg peak areas.

Wiese C, Dray E, Groesser T, San Filippo J, Shi I, Collins DW, Tsai MS, Williams GJ, **Rydberg B**, Sung P, **Schild D**. Promotion of homologous recombination and genomic stability by RAD51AP1 via RAD51 recombinase enhancement. Mol Cell. 2007 Nov 9;28(3):482-90. PMID: 17996711

Homologous recombination (HR) repairs chromosome damage and is indispensable for tumor suppression in humans. RAD51 mediates the DNA strand-pairing step in HR. RAD51 associated protein 1 (RAD51AP1) is a RAD51-interacting protein whose function has remained elusive. Knockdown of RAD51AP1 in human cells by RNA interference engenders sensitivity to different types of genotoxic stress, and RAD51AP1 is epistatic to the HR protein XRCC3. Moreover, RAD51AP1-depleted cells are impaired for the recombinational repair of a DNA double-strand break and exhibit chromatid breaks both spontaneously and upon DNA-damaging treatment. Purified RAD51AP1 binds both dsDNA and a D loop structure and, only when able to interact

with RAD51, greatly stimulates the RAD51-mediated D loop reaction. Biochemical and cytological results show that RAD51AP1 functions at a step subsequent to the assembly of the RAD51-ssDNA nucleoprotein filament. Our findings provide evidence that RAD51AP1 helps maintain genomic integrity via RAD51 recombinase enhancement.

Huang B, Brennan KM, Budinger TF, Maltz JS. Assessment of endothelial function in the radial artery using inhaled albuterol. Conf Proc IEEE Eng Med Biol Soc. 2007;1:3629-31 PMID: 18002782

Endothelial dysfunction is an early indicator of developing atherosclerosis and is a strong predictor of future heart attack and stroke. At present, evaluation of endothelial function (EF) (specifically, EF mediated by nitric oxide, NO) is too technically difficult to form part of a routine clinical examination. Non-invasive methods that measure NO-dependent EF in arteries make use of a 4-5 minute blood pressure cuff occlusion of the arm in order to induce reactive hyperemia (RH) upon cuff release. The increased blood flow that results from the RH stimulates the endothelial cells to release NO and relax the surrounding vascular smooth muscle. The magnitude of the change in arterial caliber or stiffness provides a measure of EF. The cuff occlusion is uncomfortable and inflation and release inevitably move the arm, increasing the technical difficulty of obtaining reliable measurements. In Beta₂-adrenergic agonist albuterol induces NO-mediated vasorelaxation in resistance vessels of humans. We examine, for the first time, the effect of albuterol on conduit vessels (radial artery) by measuring changes in the transit times of artificial pulses observed after inhalation of albuterol. We conclude that albuterol is able to relax the radial artery and that this correlates with the effects of RH ($r=0.62$, $p=0.04$). However, the response to a dose of 360 μ -g is smaller and more variable when compared to the response to RH-based stimulus.

Jagust WJ, Zheng L, Harvey DJ, Mack WJ, Vinters HV, Weiner MW, Ellis WG, Zarow C, Mungas D, Reed BR, Kramer JH, Schuff N, Decarli C, Chui HC. Neuropathological basis of magnetic resonance images in aging and dementia. Ann Neurol. 2007 Dec 21 [Epub ahead of print] PMID: 18157909

OBJECTIVE: Magnetic resonance (MR) imaging is used widely for assessment of patients with cognitive impairment, but the pathological correlates are unclear, especially when multiple pathologies are present. METHODS: This report includes 93 subjects from a longitudinally followed cohort recruited for the study of Alzheimer's disease (AD) and subcortical cerebrovascular disease (CVD). MR images were analyzed to quantify cortical gray matter volume, hippocampal volume, white matter hyperintensities, and lacunes. Neuropathological examination quantified CVD parenchymal pathology, AD pathology (defined as Consortium to Establish a Registry for Alzheimer's Disease scores and Braak and Braak stage), and hippocampal sclerosis. Subjects were pathologically classified as 12 healthy control subjects, 46 AD, 14 CVD, 9 mixed AD/CVD, and 12 cognitively impaired patients without significant AD/CVD pathology. Multivariate models tested associations between magnetic resonance and pathological findings across the entire sample. RESULTS: Pathological correlates of cortical gray matter volume were AD, subcortical vascular pathology, and arteriosclerosis. Hippocampal volume was related to AD pathology and hippocampal sclerosis, and the effects of hippocampal sclerosis were greater for subjects with low levels of AD pathology. White matter hyperintensities were related to age and to white matter pathology. Number of MRI lacunes was related to subcortical vascular pathology. INTERPRETATION: In this clinical setting, the presence of lacunes and white matter

changes provide a good signal for vascular disease. The neuropathological basis of MR defined cerebral cortical and hippocampal atrophy in aging and dementia is complex, with several pathological processes converging on similar brain structures that mediate cognitive decline.

Salo R, **Nordahl TE**, Leamon MH, Natsuaki Y, Moore CD, Waters C, Carter CS. Preliminary evidence of behavioral predictors of recurrent drug-induced psychosis in methamphetamine abuse. Psychiatry Res. 2008 Jan 15;157(1-3):273-7. Epub 2007 Oct 24. PMID: 17928066

The goal of this study was to examine behavioral characteristics of currently drug-abstinent methamphetamine (MA)-dependent subjects (n=39) who experienced psychotic symptoms associated with MA abuse. All participants completed the Wender Utah Rating Scale (WURS), which retrospectively assesses Attention Deficit Hyperactivity Disorder-relevant childhood behaviors. The results suggest the existence of possible behavioral markers reflecting an early cognitive vulnerability to the development of frequent MA-induced psychotic symptoms as well as increased vulnerability associated with a family history of psychiatric illness.