



LIFE SCIENCES DIVISION E-NEWSLETTER

January/February, 2010

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DOE scientific focus area notes

Low Dose Radiation Research

PNNL and Berkeley Lab Discuss Integration into Regulatory Policy

Paul Locke from the Johns Hopkins Bloomberg School of Public Health presented the Life Sciences and Genomics Divisions Seminar on January 7, 2010 entitled "Incorporating Information from the DOE Low Dose Research Program into Regulatory Decision-making: Challenges and Opportunities." His seminar, hosted by **Amy Kronenberg**, examined the challenges of integrating Berkeley Lab's Life Sciences low dose science into regulatory policy.

Locke's presentation considered just how much science is required to regulate from scientific and legal perspectives. He highlighted the concept of "weight of evidence" in the regulatory and legal communities, and provided examples of different approaches to provide such assessments. The evolution of risk assessment methodologies was also presented with reference to a series of reports from the National Academy of Sciences. Also illustrated was the 1980 U.S. Supreme Court ruling on the right of Federal agencies to regulate at the frontiers of science, provided they substantiate their conclusions based on the available data. In addition, Locke provided examples of the divergence of the approaches agencies used to set exposure standards. Three challenges were put forth by Locke for consideration by the audience: the development of a systems biology model to consider low dose effects, how to consider population susceptibility in molecular and mechanistic studies, and how to integrate the results of such studies with radiation epidemiology.



Bill Morgan, leader of the DOE Low Dose Scientific Focus Area research at the Pacific Northwest National Laboratory (PNNL), also attended the seminar and joined Locke, **Joe Gray**, and Berkeley Lab Low Dose Scientific Focus Area members in a brainstorming session later that afternoon. The discussion was centered on the integration of science between the Berkeley Lab and PNNL initiatives, with the goal to help move the basic science forward in a manner that will help address the challenges proposed by Locke.

Amy Kronenberg, 1/10

Low Dose Publication on New Computational Method

Life Sciences scientists **Ju Han**, Hang Chang, **Paul Yaswen**, **Bahram Parvin** and colleagues have published an article in the *IEEE/ACM Translational Computational Biology Bioinformatics* on a new computational method that demonstrates that multidimensional representations of cell-by-cell phenotypes improve predictive and visualization capabilities among different radiation qualities, and identify hidden variables. This technology has been developed for high content screening of important membrane-bound macromolecules that could be altered as a result of low dose radiation. Integrity of membrane-bound macromolecules is an integral component of tissue architecture.

Abstract: Cell membrane proteins play an important role in tissue architecture and cell-cell communication. We hypothesize that multidimensional characterization of the distribution of cell membrane proteins, on a cell-by-cell basis, enable improved classification of treatment conditions (e.g., low dose radiation, therapeutic agents) and identify important characteristics that can otherwise be hidden. We have developed a series of computational steps to 1) delineate cell membrane protein signals and associate them with the corresponding nucleus; 2) compute a coupled representation of the multiplexed DNA content with membrane proteins; 3) rank computed features associated with such a multidimensional representation; 4) visualize selected features for comparative evaluation through heatmaps; and 5) discriminate between treatment groups in an optimal fashion. The novelty of our method is in the segmentation of the membrane signal and the multidimensional representation of phenotypic signature on a cell-by-cell basis. To test the utility of this method, the proposed computational steps were applied to images of cells that have been irradiated with different radiation qualities in the presence and absence of other small molecules. We demonstrate that multidimensional representations of cell-by-cell phenotypes improve predictive and visualization capabilities among different treatment groups, and identify hidden variables.

Han J, Chang H, Andarawewa K, Yaswen P, Barcellos-Hoff MH, Parvin B. Multidimensional profiling of cell surface proteins and nuclear markers. *IEEE/ACM Transactions on Computational Biology and Bioinformatic*, 2010 Jan-Mar;7(1):80-90. PMID: 20150670

CG, 1/10

GTL-Genomics

Key Protein in ABA Signaling Pathway Confirmed

Plants are capable of responding to environmental stresses such as drought or extreme temperatures through bonafide hormone signaling pathways. One such plant signaling pathway utilizes the small molecule abscisic acid (ABA) which adapts a plant during water-stressed environmental changes. ABA can induce seed dormancy, plant development, provide protection from drought or protection from abrupt salinity changes. The protein receptors involved in the ABA pathway have remained elusive until recently. In a collaboration between Life Sciences scientist **Robert Rambo** at Beamline 12.3.1 of the Advanced Light Source, and Noriyuki Nishimura and co-workers of The Scripps Research Institute, the structural details of ABA binding by its cognate protein receptor, pyrabactin resistance 1 (PYR1), have been determined using a combination of small angle X-ray scattering (SAXS), multi-angle light scattering and X-ray crystallography. The crystallographic structure reveals a homodimeric protein assembly with a large ABA binding cavity. SAXS studies suggest the receptor protein switches between “open-lid” and “closed-lid” conformations with ABA demonstrating hormone induced structural changes in PYR1. Furthermore, in plant studies using site-directed PYR1 mutants designed to disrupt hormone binding lose ABA-triggered interactions with an additional protein partner, the type 2C protein phosphatase. This result clearly demonstrates disruption of the hormone signaling pathway in the plant and further strengthens the notion that PYR1 is a key protein in the ABA signaling pathway. The research was published in the December issue of *Science*:

Nishimura N, Hitomi K, Arvai AS, Rambo RP, Hitomi C, Cutler SR, Schroeder JI, Getzoff ED. Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. *Science*. 2009 Dec 4;326(5958):1373-9.

Rob Rambo, 1/10

Radiochemistry & Instrumentation

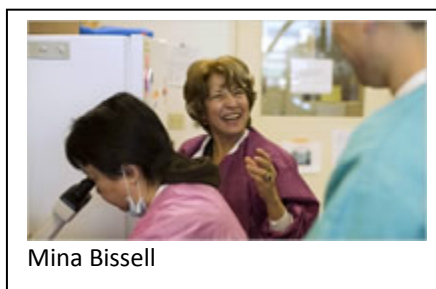
SFA Welcomes Addition of New Team

A new team, led by Marit Nilsen-Hamilton, was added to the Berkeley Lab Scientific Focus Area (SFA) entitled Radiotracer Imaging Technologies for Plant, Microbial, and Environmental Systems. Nilsen-Hamilton is a Professor of Biochemistry and Molecular Biology at Iowa State University, and her group at the DOE Ames Laboratory adds a strong Molecular Biology core to the SFA, which already has cores in Instrumentation, Radiochemistry, Data Analysis, Scintillators, Radiotracer Resources, and Probe Development. Planned areas of research are studying subsurface microbial colonies for environmental remediation and processes related biofuels development.

William Moses, 1/10

Scientific & divisional news

Lab Researchers Make Pages of *Chronicle*, *Mercury News* and *New York Times*



A *New York Times* article chronicled life scientist **Mina Bissell** (middle) and her groundbreaking research on cancer tumors and their cellular environments.

For 20 years Mina Bissell struggled for acceptance of what seemed a radical idea: Cancer involves not just gene mutations, but an interaction between rogue cells and surrounding tissue. Now more researchers are studying tumors in their cellular environments, a major shift in thinking about why cancer occurs and how to stop it. [*New York Times*, 12/28/09] More>

http://www.nytimes.com/2009/12/29/health/research/29cancer.html?_r=1

Today at Berkeley Lab, CG, 1/5/10

PNAS Article: Life's Cargo Carrier of the Cells Moves Like a Seesaw

Life's smallest motor, a protein that shuttles cargo within cells and helps cells divide, does so by rocking up and down like a seesaw, according to research conducted by scientists at Berkeley Lab and Brandeis University. The researchers created high-resolution snapshots of a protein motor, called kinesin, as it walked along a microtubule, which are tube-shaped structures that form a cell's "skeleton." The result is the closest look yet at the structural changes kinesin proteins undergo as they ferry molecules within cells.

"We see for the first time how kinesin's atomic-scale moving parts allow it to pull itself and its cargo along a microtubule," says **Ken Downing**, a biophysicist with Berkeley Lab's Life Sciences Division. He conducted the research with postdoctoral fellow Charles Sindelar, now at Brandeis University.

More ><http://newscenter.lbl.gov/feature-stories/2010/02/17/kinesin-seesaw/>

Sindelar CV, Downing KH. An atomic-level mechanism for activation of the kinesin molecular motors. *Proceedings of the National Academy of Sciences U S A*, 2010 Feb 16. [Epub ahead of print]

Today at Berkeley Lab, CG, 2/18/10

Janecek's Optical Model Accepted by GEANT4

In 2008 **William Moses**, **Martin Janecek** and **David Wilson** of the Life Sciences Division's Department of Radiotracer Development and Imaging Technology designed a [novel instrument](#) for measuring how light is reflected inside a scintillating crystal. Janecek used the instrument to collect data that allowed him to accurately model the performance of scintillator-based radiation detectors. In January, he was notified that his optical model, which outperforms state-of-the-art programs, has been incorporated into the latest release of the powerful [GEANT4 computational toolkit](#). GEANT, which stands for Geometry and Tracking, was originally developed at the European Organization for Nuclear Research (CERN) for simulating the passage of particles through matter in high-energy physics but has found wide use in numerous scientific applications, including nuclear medicine.

Today at Berkeley Lab, SD, 2/18/10



Discovery of Two New High-Performance Scintillators for National Security

One major problem in National Security is finding hidden nuclear threats such as uranium and plutonium in the millions of tons of cargo that are transported each year during the normal course of commerce. This challenge is made more difficult by the background radiation from large amounts of slightly radioactive cargo that are not a threat, such as bananas, granite, and kitty litter. Bananas contain potassium-40 while granite and kitty litter contain thorium. In addition, there are medical radioisotopes that must be transported without hindrance. So it is not sufficient to merely detect radiation; a threat detection system must be able to identify the specific radioactive isotopes that are producing the radiation. While there are many types of radiation, only gamma rays have the penetrating power to be detectable outside of trucks or shipping containers. Fortunately, different radioactive isotopes emit gamma rays of different energies, and a measurement of those energies provides a "fingerprint" that is characteristic of each isotope. This leads to three major challenges for the detector: (1) it must be dense enough and large enough to efficiently stop the energetic gamma rays that emerge from the container, (2) it must be inexpensive enough to be deployed at the thousands of inspection locations where it is needed, and (3) it must be able to measure gamma ray energies with enough accuracy to distinguish security-threat isotopes from commonly shipped radioactive isotopes.

The most commonly used radiation detector for gamma rays is the scintillator that works by absorbing gamma rays and emitting flashes of light whose intensity is proportional to the energy deposited by each gamma ray. Sensitive photodetectors and electronic circuits detect these flashes. The scintillator that is most commonly used for rapid cargo screening is an organic plastic that is relatively inexpensive but the gamma rays only deposit a random fraction of their energy (i.e. they are scattered rather than absorbed). Consequently, the plastic scintillator can detect gamma rays but not measure their energies. Whenever the plastic scintillators detect radiation, time-consuming secondary screening is needed to identify the radioisotope. This secondary screening could be avoided if the scintillator had the ability to stop the gamma rays and accurately record their energies. Sodium iodide is a scintillator that has good stopping power for gamma rays and can measure their energies, but not accurately enough for rapid

isotope identification. A newer scintillator, lanthanum bromide, has good stopping power and much better energy accuracy than sodium iodide, but after years of development sufficiently large crystals are still too expensive.

Researchers at Berkeley Lab have discovered two new scintillators that could improve this situation: barium bromide and cesium barium iodide. Both scintillators have good stopping power and energy resolution. While these compounds were previously known, the Berkeley Lab team was the first to grow crystals and measure their response to gamma rays. Work is continuing to develop low-cost crystal production at Berkeley Lab and with an industrial partner. These discoveries were made in an ongoing project (funded by the Department of Homeland Security) to synthesize and test hundreds of compounds and find improved scintillators. The first papers on these new scintillators were recently published in *Nuclear Instruments and Methods in Physics Research*.

E. D. Bourret-Courchesne, **G. Bizarri**, **S. M. Hanrahan**, **G. Gundiah**, Z. Yan, and **S. E. Derenzo**, "BaBr:Eu²⁺, a new bright scintillator" *Nuclear Instruments and Methods in Physics Research A*, vol. 613, pp. 95-97, 2009.

E. D. Bourret-Courchesne, **G. Bizarri**, **R. Borade**, Z. Yan, **S. M. Hanrahan**, **G. Gundiah**, **A. Chaudhry**, **A. Canning**, and **S. E. Derenzo**, "Eu²⁺-doped Ba₂CsI₅, a new high-performance scintillator," *Nuclear Instruments and Methods in Physics Research A*, vol. 612, pp. 138-142, 2009.

Stephen Derenzo, 2/10

Why You Should Step Up Your Workout

[Wall Street Journal] To **Paul Williams**, spurring more exercise out of the half of Americans who are already active is just as important as coaxing the sedentary off the sofa. In Williams' study of more than 100,000 runners over nearly 20 years, stepped up exercise was found to have some powerful benefits. But his research is controversial. While Williams is well respected by other exercise scientists, he is shunned by those in the public-health field. Public-health officials also worry that touting Williams's research could discourage the sedentary from doing any exercise at all, or lure them off the couch with goals too lofty to engender success. More>

<http://online.wsj.com/article/SB10001424052748704350304574638550059084962.html>

Today at Berkeley Lab, 1/6/10

Life Sciences Welcomes High School Seniors at Seminar

A group of 18 seniors of The American High School in Fremont attended the Life Sciences and Genomics Divisions Seminar of Zena Werb of the University of California, San Francisco, on January 5, 2010, as part of their Physics Honors course. Their class assignment was to attend a professional-level scientific lecture/seminar (not necessarily related to physics) at an esteemed institution and to write a report on what they have learned. The seminar of Werb, who presented on "Transcriptional Regulation of Breast Cancer Progression and Metastasis," was extremely well received.



The seminar was selected by Senior Alexander Prucha, who was already familiar with the Lab and had done an internship with Life Sciences scientist Frank Chen the past summer, via the Berkeley Lab Internships for Precollegiate Scholars (BLIPS) program, administered by the Center for Science and Engineering Education (CSEE). He worked on research related to expression of HER-2 antibody in transfected E. coli cells, to use the antibody in a cancer-detection nanoparticle. He commented: "it was great for me to be back at the Lab," and "It was wonderful that Dr. Werb took the time to explain some of the more complex topics in terms that we could better understand; We all walked away having understood and learned something new and exciting."

The students were welcomed by seminar host **Joe Gray** and after the seminar pictures were taken with the students, Gray, Werb and colleague **Mina Bissell**.

Today at Berkeley Lab, CG, 1/15/10

Life Sciences Hosts NCI Chief Jeffrey Green

The Life Sciences Division is currently hosting Jeff Green, a scientist of the National Cancer Institute (NCI), on sabbatical for 6 months with **Joe Gray** and **Mina Bissell**. Green, Chief of the Transgenic Oncogenesis and Genomics Section, Laboratory of Cancer Biology and Genetics, is an expert on animal models of mammary cancer. Using cross-species genomic analyses, he is interested in identifying which models best represent specific sub-types of human breast cancer and potential novel therapeutic targets in collaboration with the Gray lab. Genetically-engineered mouse models offer important opportunities to test drugs and combination therapies that can be translated to the clinic. Green's lab also studies the regulation of tumor cell dormancy by the extracellular environment and will work with the Bissell lab to advance this area of research. The Green lab has developed novel models to explore triggers that activate dormant tumor cells to awaken and proliferate into recurrent, metastatic disease.

CG, 1/10

Torok Leads Food Safety Workshop in Jordan

Last month Life Sciences scientist **Tamas Torok** led a two-week advanced food safety workshop focusing on food- and water-borne pathogens for 15 Iraqi scientists in Amman, Jordan. The aim of the DOE and Sandia National Laboratory organized workshop was to provide advanced-level training for Iraqi scientists in the detection and identification of food- and water-borne pathogenic microorganisms. Given Torok's background in food microbiology, human pathogens, and biosafety/biosecurity, he was asked to design and lead the program, which was held on the campus of the Royal Scientific Society in Amman. The teaching material was based on the most recent developments in food microbiology, international food safety trends, and state-of-the-art detection methods but also responded to the needs of the participants. The mornings were spent in the laboratory, the afternoons in the classroom. In two weeks, Torok and his team covered the ten "most wanted" food-borne pathogens (FDA) and discussed ISO accreditation procedures, risk assessment modeling, and surveillance and predictive food microbiology. Torok also had the opportunity to visit Jordanian institutes while there.

Tamas Torok, 1/10



Making Room for a Lab That's Growing; Life Scientist Leads Committee

People are most productive when they have some measure of physical proximity and contact with their co-workers. Our personal workspace needs to be flexible, energy efficient and pleasant to occupy, and it should encourage collaboration and dialogue. These are the basic principles driving a new strategic approach to office and lab space, as the Lab prepares to face a problem that few other employers have these days — an influx of employees, and not enough space to put everyone. In the coming 24 months, workstations must be found for up to 500 employees as well as 20,000 square feet of additional laboratory space.



Sudar (left), SPAC chairperson

Lab management named Deputy Chief Operating Officer Anita Gursahani as the Berkeley Lab Space Manager and chartered the Berkeley Lab Space Advisory Committee (known as SPAC). Its members include four scientists — Ali Belkacem (Chemical Sciences), Ernie Majer (ESD), Natalie Roe (Physics) and **Damir Sudar** (Deputy of

Technology, Life Sciences) as chairperson — and two individuals from Operations — Helen Cademartori and Rich McClure — as well as Gursahani. Their first order of business is to recommend to management policies and procedures for managing space, including a metric, or approximately how much space, on average, should be allocated per person, within three months. [More>](#)

<http://www.lbl.gov/publicinfo/newscenter/tab/2010/february/02-16-10/jump-photos.html>

Today at Berkeley Lab, CG, 2/16/10

Building 74 Modernization Project Passes CD-3B Milestone

On February 2 and 3, a team of reviewers from other national labs conducted a peer review of Berkeley Lab's design documents and project plans prepared for the modernization of the Life Sciences laboratory Building 74. Based on the Peer Review team recommendations, DOE has formally made a decision known as CD-3B, which gives Berkeley Lab the green light to start construction work in late March. Earlier phases of this critical project included the seismic retrofit of the building, which was completed in September 2009, and the interior demolition work which was completed in February. The modernization work, which is part of the SLI-funded Seismic Life Safety, Modernization, and Replacement of General Purpose Buildings Phase 2 Project, is on schedule and expected to be complete in late 2011.

Damir Sudar, 2/10

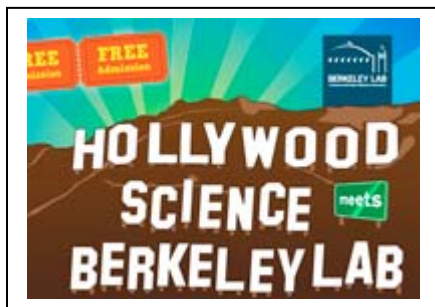


Increase in Technology Royalties for FY09

Last month, Lab Director Paul Alivisatos recognized researchers who have earned royalties through the successful commercialization of their technologies. Out of the Lab's \$3.8 million in licensing income for FY09, an 18-percent increase from the previous year, over \$1.2 million went directly to 128 scientists and authors from the Lab. The Technology Transfer Department negotiates the right to use Berkeley Lab inventions with organizations, from Fortune 500 firms to small start-ups. Offering exclusive or non-exclusive rights to the technologies ensures that the Lab's inventions are successfully commercialized and, ultimately, meet society's needs. Go here for full story and picture of royalty recipients that

include life scientists **John Bielicki, Dan Callahan, Judith Campisi, Frank Chen, Jamie Eberling, Yoshinori Kohwi, Terumi Kohwi-Shigematsu, Bahram Parvin, Martha Stampfer, and Uli Weier** . More>
<http://www.lbl.gov/publicinfo/newscenter/tab1/2010/february/02-26-10/tech-transfer-jump.html>
Today at Berkeley Lab, 2/26/10

Science on the Silver Screen Topic of ‘Theater’ Panel Discussion; Dernburg Participates



Science on the big screen was the topic of the Science at the Theater program on February 3 on the Roda Stage at the Berkeley Rep theatre. Sidney Perkowitz, author of *Hollywood Science*, moderated a panel of Lab researchers, including climate scientist Bill Collins, physicist Richard Muller, and life scientist **Abby Dernburg**. The following evening, the Lab hosted a science café featuring the creator and star of the popular SyFy *show* Eureka. Attendees were invited to submit a plot line for the show involving the Lab and could win front row seats and an introduction to the

Eureka guests.

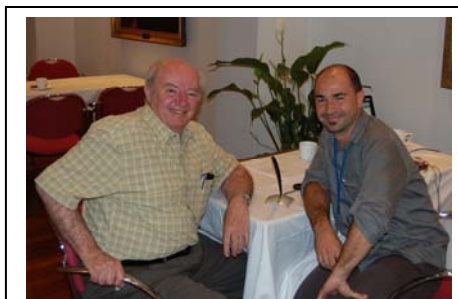
Today at Berkeley Lab, 1/26/10

Meet a Scientist: Sylvain Costes Broadcasting Research in Radiation Sciences

Life Sciences scientist **Sylvain Costes'** voice can be heard regularly on the Radiation Research Podcast, the podcast for the scientific journal *Radiation Research* of the Radiation Research Society (RRS). The inception of this Podcast goes back to late 2005/early 2006 in New Jersey, when three postdoctoral fellows, Manuela Buonanno, Badri Pandey and Massimo Pinto, were trying to revive the readership of the *Scholar In Training Newsletter* of the RRS. This initiative, inspired by the Nature Podcast, was presented at the Philadelphia 2006 RRS meeting, at which point Sylvain Costes joined the team.

The team has now grown to 8 volunteers with Massimo Pinto and Sylvain Costes conducting the majority of the interviews over the years, all available via the RRS website at <http://www.radres.org/podcast/>. Besides interviewing, Costes also edits the recordings, helps managing the website and is otherwise passionately involved in keeping the podcasts coming. With some initial start up funds from RRS, the podcasts were transferred from the Berkeley Lab server, maintained by Costes, to the RRS website server. Unfortunately, the podcast team often struggles to secure funding for the maintenance of the server.

Interviewees are selected via consensus, with votes submitted based on the selection of RRS publications or awards received in the area of radiation research. But when an opportunity arises to sit down with an interesting scientist at a conference or meeting, Costes does not hesitate and takes the opportunity to interview a “hot” scientist. Most people who are asked to be interviewed respond very positively, Costes says, and “we prefer to interview in person”. The interviews follow a simple frame of covering the what, why, and relevance of a research topic. When asked who he would like to sit down with at his next scientific meeting, his eyes light up and he recalls with great passion interviews of the past, such as



Costes (right) with interviewee Joel Bedford (Colorado State University) at the “Heavy Ions in Therapy and Space Symposium 2009,” in Cologne, Germany.

the interview with a trio of scientists, Mary Helen Barcellos-Hoff, Jack Little, and Edouard Azzam, whom he asked to give their definition of the “controversial” radiation-induced bystander effect. This round table discussion was “quite light, more dynamic and really cool and fun to lead,” Costes said. He also mentions the interview with NASA program manager Frank Cucinotta, who gave insights in the NASA mission. Among other memorable interviews, Costes recalls the interview with Rainer Sachs, mathematician at the University of California, Berkeley and former postdoc advisor of Costes back in 2001 when they used their knowledge of physics and mathematics to model DNA damage from ionizing radiation.

Still, Costes looks forward to more exciting interviews in the future. One person he would enjoy interviewing on a one-on-one basis is Mary Helen Barcellos-Hoff, whom he worked with in Life Sciences but is currently mainly affiliated with the New York University Medical Center. “She was part of the trio I recently interviewed but I think it would be great to have her on the record regarding her thoughts about radiation carcinogenesis. She has a very distinct view from the general approach by considering that damage to the extra cellular matrix is probably as important or maybe more important than the DNA damage generated by radiation as far as inducing cancer years after exposure. This was not considered 20 years ago and she has radically changed the radiation biology field in that sense...”

As part of the Low Dose Scientific Focus Area in Life Sciences, Costes studies the influence of chromatin organization on radiation-induced DNA damage. “The global changes observed in the chromatin following exposure to ionizing-radiation may help explaining emerging phenotypes at the cell population level observed hours to days after the perturbation. For example, controversial phenomena such as adaptive response or genomic instability may be modulated by these changes”. And although he already has his colleagues **Andrew Wyrobek** and **Francesco Marchetti** on the record, he hopes to be able to capture more Life Sciences research in a podcast in the future.

More about Costes’ work>

http://www.lbl.gov/lsd/People_&_Organization/Scientific_Staff_Directory/Costes_Lab.html

CG, 1/10

Awards

Berkeley Lab Awarded \$12.8 Million in Stimulus Funds for Health Research

Lawrence Berkeley National Laboratory has been awarded \$12.8 million in American Recovery and Reinvestment Act funding by the National Institutes of Health (NIH) for research into cancer, neurodegenerative diseases, radioactive decontamination and a variety of other health conditions.

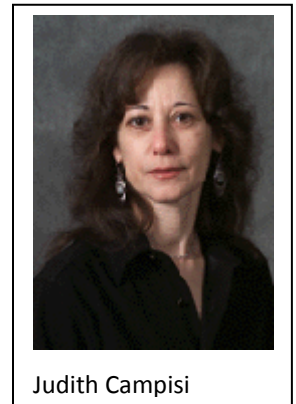
“The Recovery Act grants from NIH have allowed us to create quite a few new positions for scientists, technicians, research associates and postdoctoral fellows, as well as retain some jobs,” said **Joe Gray**, Associate Laboratory Director for Life Sciences. “We’re helping to train the nation’s next generation of scientists while also doing important research in critical areas of human health that we may not have been able to do without these funds.” News Release>

<http://newscenter.lbl.gov/news-releases/2010/01/19/berkeley-lab-awarded-12-8-million-in-stimulus-funds-for-health-research/>

Berkeley Lab News Release, 1/19/10

Campisi to Receive Ipsen Longevity Prize 2010

Life Sciences scientist **Judith Campisi**, internationally known for her work on cellular aging and “telomeres,” has been awarded the Ipsen Longevity Prize 2010. Ipsen, an innovation-driven global specialty pharmaceutical group - based in France - which awards several prizes to encourage research, recognized Campisi for her outstanding contributions to understanding aging. Campisi's winning research was the discovery of a molecular link between a tumor suppressive response to DNA damage (cellular senescence) and inflammation, which underlies virtually all age-related pathologies, including (ironically) cancer. The findings not only have broad implications for how and why we age, but suggest novel strategies for mitigating the pro-aging effects of DNA damage.



Campisi's research focuses on understanding the cellular and molecular biology of aging, specifically by exploring the causes and consequences of cellular senescence (when cells are no longer able to divide) and cell death, and the effects of DNA damage and repair on premature aging and cancer-prone syndromes. Campisi, who also holds a position at the Buck Institute of Age Research, will accept the award later this year in New Orleans.

CG, 1/10

Recent publications

Bielicki JK, Zhang H, Cortez Y, Zheng Y, Narayanaswami V, Patel A, Johansson J, Azhar S. A new HDL mimetic peptide that stimulates cellular cholesterol efflux with high efficiency greatly reduces atherosclerosis in mice. *Journal of Lipid Research*, 2010 Jan 14. [Epub ahead of print] PMID: 20075422

Here we report the creation of a single-helix peptide (ATI-5261) that stimulates cellular cholesterol efflux with Km molar efficiency approximating native apolipoproteins. Anti-atherosclerosis activity of ATI-5261 was evaluated in LDLR^{-/-} and apoE^{-/-} mice ~5-7 months of age, following 13-18 weeks on high-fat western-diet. Treatment of fat-fed LDLR^{-/-} mice with daily ip injections of ATI-5261 (30 mg/kg) for 6 weeks reduced atherosclerosis by 30%, as judged by lesion-area covering the aorta (7.9+/-2 vs.11.3+/-2.5% control, p=0.011) and lipid-content of aortic sinus plaque (25+/-5.8 vs. 33+/-4.9% control, p=0.014). In apoE^{-/-} mice, the peptide administered 30 mg/kg ip on alternate days for 6 weeks reduced atherosclerosis by ~45% (lesion area = 15+/-7 vs. 25+/-8% control, p=0.00016; plaque lipid-content = 20+/-6 vs. 32+/-8% control, p<0.0001). Similar reductions in atherosclerosis were achieved using ATI-5261:POPC complexes. Single ip injection of ATI-5261 increased reverse cholesterol transport from macrophage foam-cells to feces over 24-48 h. In summary, relatively short-term treatment of mice with the potent cholesterol efflux peptide ATI-5261 reduced substantial atherosclerosis. This was achieved using an L-amino acid peptide, in the presence of severe hypercholesterolemia/HFWD, and did not require daily injections or formulation with phospholipids when administered via ip injection.

Bizarri G, Moses WW, Singh J, Vasil'ev AN, Williams RT. The role of different linear and non-linear channels of relaxation in scintillator non-proportionality. *Journal of Luminescence (Special Issue)*, 2009 Dec;129(12):1790-1793.

The non-proportional dependence of a scintillator's light yield on primary particle energy is believed to be influenced crucially by the interplay of non-linear kinetic terms in the radiative and non-radiative decay of excitations versus locally deposited excitation density. A calculation of energy deposition, $-dE/dx$, along the electron track for NaI is Presented for an energy range from several election-volt to 1 MeV. Such results can be used to specify an initial excitation distribution, if diffusion is neglected. All exactly solvable two-channel (exciton and hole(electron)) model containing 1st and 2nd order kinetic terms is constructed and used to illustrate important features seen in non-proportional light-yield curves, including a dependence on pulse shaping (detection gate width).

The efficiency of two biomass pretreatment technologies, dilute acid hydrolysis and dissolution in an ionic liquid, are compared in terms of delignification, saccharification efficiency and saccharide yields with switchgrass serving as a model bioenergy crop. When subject to ionic liquid pretreatment (dissolution and precipitation of cellulose by anti-solvent) switchgrass exhibited reduced cellulose crystallinity, increased surface area, and decreased lignin content compared to dilute acid pretreatment. Pretreated material was characterized by powder X-ray diffraction, scanning electron microscopy, Fourier transform infrared spectroscopy, Raman spectroscopy and chemistry methods. Ionic liquid pretreatment enabled a significant enhancement in the rate of enzyme hydrolysis of the cellulose component of switchgrass, with a rate increase of 16.7-fold, and a glucan yield of 96.0% obtained in 24h. These results indicate that ionic liquid pretreatment may offer unique advantages when compared to the dilute acid pretreatment process for switchgrass. However, the cost of the ionic liquid process must also be taken into consideration.

Bourret-Courchesne E. D., **Bizarri G.**, Hanrahan S. M., **Gundiah G.**, Yan Z., and **Derenzo S. E.** BaBrI:Eu²⁺, a new bright scintillator. *Nuclear Instruments and Methods in Physics Research A*, vol. 613, pp. 95-97, 2009.

See also News Highlight, page 5.

The scintillation properties of BaBrI:Eu²⁺ are reported. Crystals were produced by the vertical Bridgman technique in a sealed quartz ampoule. Excellent scintillation properties were measured. A light yield of 81,000 +/- 3000 photons per MeV (ph/MeV) of absorbed gamma-ray energy was measured. An energy resolution (FWHM over peak position) of 4.8 +/- 0.5% was observed for the 662 keV full absorption peak. Pulsed X-ray luminescence measurements show two exponential decay components of 297 and 482 ns with a contribution to the total light output of 23% and 77%, respectively. Under X-ray and UV excitation, the emission corresponds to a broadband center at 413 nm. These initial values make BaBrI:Eu²⁺ one of the brightest and the fastest known Eu²⁺-doped scintillators.

Bourret-Courchesne E. D., **Bizarri G.**, **Borade R.**, Yan Z., Hanrahan S. M., **Gundiah G.**, **Chaudhry A.**, **Canning A.**, and **Derenzo S. E.** Eu²⁺ -doped Ba₂CsI₅, a new high-performance scintillator. *Nuclear Instruments and Methods in Physics Research A*, vol. 612, pp. 138-142, 2009.

See also News Highlight, page 5.

The crystal growth and scintillation properties of Ba₂(CsI)₅:Eu²⁺ are reported. Crystals were produced by the vertical Bridgman technique in a sealed quartz ampoule. Ba₂(CsI)₅:Eu²⁺ presents excellent scintillation properties. An estimated light yield of 97,000 +/- 5,000 photons per MeV (ph/MeV) of absorbed gamma-ray energy was measured. An energy resolution (FWHM over peak position) of 3.8 +/- 0.3% was observed for the 662 keV full absorption peak. Pulsed X-ray luminescence measurements show a relatively complex time response with four exponential decay components of 48,383, 1500 and 9900 ns with a contribution to the total light output of 1%, 26%, 68% and 25%, respectively. Under X-ray and UV excitation, the emission corresponds to a broadband centered at 2.85 eV. First principles calculations show strong localization of the excited state on the Eu site. Ba₂(CsI)₅:Eu²⁺ has a density of about 5

g/cm³). These first reported scintillation properties make Ba(2)CsI(5):Eu²⁺ a very high-performance scintillator.

Han J, **Chang H**, Andarawewa K, **Yaswen P**, Barcellos-Hoff MH, **Parvin B**. Multidimensional profiling of cell surface proteins and nuclear markers. *IEEE/ACM Transactions on Computational Biology and Bioinformatic*, 2010 Jan-Mar;7(1):80-90. PMID: 20150670

Cell membrane proteins play an important role in tissue architecture and cell-cell communication. We hypothesize that segmentation and multidimensional characterization of the distribution of cell membrane proteins, on a cell-by-cell basis, enable improved classification of treatment groups and identify important characteristics that can otherwise be hidden. We have developed a series of computational steps to 1) delineate cell membrane protein signals and associate them with a specific nucleus; 2) compute a coupled representation of the multiplexed DNA content with membrane proteins; 3) rank computed features associated with such a multidimensional representation; 4) visualize selected features for comparative evaluation through heatmaps; and 5) discriminate between treatment groups in an optimal fashion. The novelty of our method is in the segmentation of the membrane signal and the multidimensional representation of phenotypic signature on a cell-by-cell basis. To test the utility of this method, the proposed computational steps were applied to images of cells that have been irradiated with different radiation qualities in the presence and absence of other small molecules. These samples are labeled for their DNA content and E-cadherin membrane proteins. We demonstrate that multidimensional representations of cell-by-cell phenotypes improve predictive and visualization capabilities among different treatment groups, and identify hidden variables.

Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annual Review of Pathology*, 2010;5:99-118. PMID: 20078217

Cellular senescence is a tumor-suppressive mechanism that permanently arrests cells at risk for malignant transformation. However, accumulating evidence shows that senescent cells can have deleterious effects on the tissue microenvironment. The most significant of these effects is the acquisition of a senescence-associated secretory phenotype (SASP) that turns senescent fibroblasts into proinflammatory cells that have the ability to promote tumor progression.

Sindelar CV, **Downing KH**. An atomic-level mechanism for activation of the kinesin molecular motors. *Proceedings of the National Academy of Sciences U S A*, 2010 Feb 16. [Epub ahead of print] PMID: 20160108

See also News Highlight, page 4.

Kinesin cytoskeletal motors convert the energy of ATP hydrolysis into stepping movement along microtubules. A partial model of this process has been derived from crystal structures, which show that movement of the motor domain relative to its major microtubule binding element, the switch II helix, is coupled to docking of kinesin's neck linker element along the motor domain. This docking would displace the cargo in the direction of travel and so contribute to a step. However, the crystal structures do not reveal how ATP binding and hydrolysis govern this series of events. We used cryoelectron microscopy to derive 8-9 Å-resolution maps of four nucleotide states encompassing the microtubule-attached kinetic cycle of a kinesin motor. The exceptionally high quality of these maps allowed us to build in crystallographically determined conformations of kinesin's key subcomponents, yielding novel arrangements of kinesin's switch II helix and nucleotide-sensing switch loops. The resulting atomic models reveal a seesaw mechanism in which the switch loops, triggered by ATP binding, propel their side of the motor domain down and thereby elicit docking of the neck linker on the opposite side of the seesaw.

Microtubules engage the seesaw mechanism by stabilizing the formation of extra turns at the N terminus of the switch II helix, which then serve as an anchor for the switch loops as they modulate the seesaw angle. These observations explain how microtubules activate kinesin's ATP-sensing machinery to promote cargo displacement and inform the mechanism of kinesin's ancestral relative, myosin.

Sun C, Su KH, Valentine J, Rosa-Bauza YT, Ellman JA, **Elboudwarej O**, Mukherjee B, Craik CS, Shuman MA, **Chen FF**, Zhang X. Time-resolved single-step protease activity quantification using nanoplasmonic resonator sensors. *ACS Nano*, 2010 Feb 23;4(2):978-84. PMID: 20121209

Protease activity measurement has broad application in drug screening, diagnosis and disease staging, and molecular profiling. However, conventional immunopeptidometric assays (IMPA) exhibit low fluorescence signal-to-noise ratios, preventing reliable measurements at lower concentrations in the clinically important picomolar to nanomolar range. Here, we demonstrated a highly sensitive measurement of protease activity using a nanoplasmonic resonator (NPR). NPRs enhance Raman signals by 6.1×10^{10} times in a highly reproducible manner, enabling fast detection of proteolytically active prostate-specific antigen (pPSA) activities in real-time, at a sensitivity level of 6 pM (0.2 ng/mL) with a dynamic range of 3 orders of magnitude. Experiments on extracellular fluid (ECF) from the pPSA-positive cells demonstrate specific detection in a complex biofluid background. This method offers a fast, sensitive, accurate, and one-step approach to detect the proteases' activities in very small sample volumes.

Hossain S, Xia P, Huang K, Descovich M, **Chuang C**, Gottschalk AR, **Roach M 3rd**, Ma L. Dose gradient near target-normal structure interface for nonisocentric CyberKnife and isocentric intensity-modulated body radiotherapy for prostate cancer. *International Journal of Radiation Oncology, Biology, Physics*, 2010 Feb 2. [Epub ahead of print] PMID: 20133073

PURPOSE: The treatment planning quality between nonisocentric CyberKnife (CK) and isocentric intensity modulation treatment was studied for hypofractionated prostate body radiotherapy. In particular, the dose gradient across the target and the critical structures such as the rectum and bladder was characterized. **METHODS AND MATERIALS:** In the present study, patients treated with CK underwent repeat planning for nine fixed-field intensity-modulated radiotherapy (IMRT) using identical contour sets and dose-volume constraints. To calculate the dose falloff, the clinical target volume contours were expanded 30 mm anteriorly and posteriorly and 50 mm uniformly in other directions for all patients in the CK and IMRT plans. **RESULTS:** We found that all the plans satisfied the dose-volume constraints, with the CK plans showing significantly better conformity than the IMRT plans at a relative greater dose inhomogeneity. The rectal and bladder volumes receiving a low dose were also lower for CK than for IMRT. The average conformity index, the ratio of the prescription isodose volume and clinical target volume, was 1.18 ± 0.08 for the CK plans vs. 1.44 ± 0.11 for the IMRT plans. The average homogeneity index, the ratio of the maximal dose and the prescribed dose to the clinical target volume, was 1.45 ± 0.12 for the CK plans vs. 1.28 ± 0.06 for the IMRT plans. The average percentage of dose falloff was $2.9\% \pm 0.8\%/mm$ for CK and $3.1\% \pm 1.0\%/mm$ for IMRT in the anterior direction, $3.8\% \pm 1.6\%/mm$ for CK and $3.2\% \pm 1.9\%/mm$ for IMRT in the posterior direction, and $3.6\% \pm 0.4\%$ for CK and $3.6\% \pm 0.4\%$ for IMRT in all directions. **CONCLUSION:** Nonisocentric CK was as capable of producing equivalent fast dose falloff as high-number fixed-field IMRT delivery.

Bowman GR, **Comolli LR**, Gaietta GM, Fero M, Hong SH, Jones Y, Lee JH, **Downing KH**, Ellisman MH, McAdams HH, Shapiro L. Caulobacter PopZ forms a polar sub-domain dictating sequential changes in pole composition and function. *Molecular Microbiology*, 2010 Feb 10. [Epub ahead of print] PMID: 20149103

The bacterium *Caulobacter crescentus* has morphologically and functionally distinct cell poles that undergo sequential changes during the cell cycle. We show that the PopZ oligomeric network forms polar ribosome exclusion zones that change function during cell cycle progression. The *parS/ParB* chromosomal centromere is tethered to PopZ at one pole prior to the initiation of DNA replication. During polar maturation, the PopZ-centromere tether is broken, and the PopZ zone at that pole then switches function to act as a recruitment factor for the ordered addition of multiple proteins that promote the transformation of the flagellated pole into a stalked pole. Stalked pole assembly, in turn, triggers the initiation of chromosome replication, which signals the formation of a new PopZ zone at the opposite cell pole, where it functions to anchor the newly duplicated centromere that has traversed the long axis of the cell. We propose that pole-specific control of PopZ function coordinates polar development and cell cycle progression by enabling independent assembly and tethering activities at the two cell poles.

Frise E, Hammonds AS, Celniker SE. Systematic image-driven analysis of the spatial *Drosophila* embryonic expression landscape. *Molecular Systems Biology*, 2010;6:345. PMID: 20087342

Discovery of temporal and spatial patterns of gene expression is essential for understanding the regulatory networks and development in multicellular organisms. We analyzed the images from our large-scale spatial expression data set of early *Drosophila* embryonic development and present a comprehensive computational image analysis of the expression landscape. For this study, we created an innovative virtual representation of embryonic expression patterns using an elliptically shaped mesh grid that allows us to make quantitative comparisons of gene expression using a common frame of reference. Demonstrating the power of our approach, we used gene co-expression to identify distinct expression domains in the early embryo; the result is surprisingly similar to the fate map determined using laser ablation. We also used a clustering strategy to find genes with similar patterns and developed new analysis tools to detect variation within consensus patterns, adjacent non-overlapping patterns, and anti-correlated patterns. Of the 1800 genes investigated, only half had previously assigned functions. The known genes suggest developmental roles for the clusters, and identification of related patterns predicts requirements for co-occurring biological functions.

Vrba L, Jensen TJ, **Garbe JC**, Heimark RL, Cress AE, Dickinson S, **Stampfer MR**, Futscher BW. Role for DNA methylation in the regulation of miR-200c and miR-141 expression in normal and cancer cells. *PLoS One*, 2010 Jan 13;5(1):e8697. PMID: 20084174

BACKGROUND: The microRNA-200 family participates in the maintenance of an epithelial phenotype and loss of its expression can result in epithelial to mesenchymal transition (EMT). Furthermore, the loss of expression of miR-200 family members is linked to an aggressive cancer phenotype. Regulation of the miR-200 family expression in normal and cancer cells is not fully understood. **METHODOLOGY/PRINCIPAL FINDINGS:** Epigenetic mechanisms participate in the control of miR-200c and miR-141 expression in both normal and cancer cells. A CpG island near the predicted miR-200c/miR-141 transcription start site shows a striking correlation between miR-200c and miR-141 expression and DNA methylation in both normal and cancer cells, as determined by MassARRAY technology. The CpG island is unmethylated in human miR-200/miR-141 expressing epithelial cells and in miR-200c/miR-141 positive tumor cells. The CpG island is heavily methylated in human miR-200c/miR-141 negative fibroblasts and miR-200c/miR-141 negative tumor cells. Mouse cells show a similar inverse correlation between DNA methylation and miR-200c expression. Enrichment of permissive histone modifications, H3 acetylation and H3K4 trimethylation, is seen in normal miR-200c/miR-141-positive epithelial cells, as determined by chromatin immunoprecipitation coupled to real-time PCR. In contrast, repressive H3K9 dimethylation marks are present in normal miR-200c/miR-141-negative fibroblasts and miR-200c/miR-141 negative cancer cells and the permissive histone modifications are absent. The epigenetic modifier drug, 5-aza-2'-deoxycytidine, reactivates miR-200c/miR-141 expression showing that epigenetic mechanisms play a

functional role in their transcriptional control. CONCLUSIONS/SIGNIFICANCE: We report that DNA methylation plays a role in the normal cell type-specific expression of miR-200c and miR-141 and this role appears evolutionarily conserved, since similar results were obtained in mouse. Aberrant DNA methylation of the miR-200c/141 CpG island is closely linked to their inappropriate silencing in cancer cells. Since the miR-200c cluster plays a significant role in EMT, our results suggest an important role for DNA methylation in the control of phenotypic conversions in normal cells.

Han J, Chang H, Giricz O, Lee GY, Baehner FL, Gray JW, Bissell MJ, Kenny PA, Parvin B. Molecular predictors of 3D morphogenesis by breast cancer cell lines in 3D culture. *PLoS Computational Biology*, 2010 Feb 26;6(2):e1000684. PMID: 20195492

Correlative analysis of molecular markers with phenotypic signatures is the simplest model for hypothesis generation. In this paper, a panel of 24 breast cell lines was grown in 3D culture, their morphology was imaged through phase contrast microscopy, and computational methods were developed to segment and represent each colony at multiple dimensions. Subsequently, subpopulations from these morphological responses were identified through consensus clustering to reveal three clusters of round, grape-like, and stellate phenotypes. In some cases, cell lines with particular pathobiological phenotypes clustered together (e.g., ERBB2 amplified cell lines sharing the same morphometric properties as the grape-like phenotype). Next, associations with molecular features were realized through (i) differential analysis within each morphological cluster, and (ii) regression analysis across the entire panel of cell lines. In both cases, the dominant genes that are predictive of the morphological signatures were identified. Specifically, PPARgamma has been associated with the invasive stellate morphological phenotype, which corresponds to triple-negative pathobiology. PPARgamma has been validated through two supporting biological assays.

Rübel O, Weber GH, Huang MY, Bethel EW, Biggin MD, Fowlkes CC, Luengo Hendriks CL, Keränen SV, Eisen MB, Knowles DW, Malik J, Hagen H, Hamann B. Integrating data clustering and visualization for the analysis of 3D gene expression data. *IEEE/ACM Transactions on Computational Biology and Bioinformatic*, 2010 Jan-Mar;7(1):64-79. PMID: 20150669

The recent development of methods for extracting precise measurements of spatial gene expression patterns from three-dimensional (3D) image data opens the way for new analyses of the complex gene regulatory networks controlling animal development. We present an integrated visualization and analysis framework that supports user-guided data clustering to aid exploration of these new complex data sets. The interplay of data visualization and clustering-based data classification leads to improved visualization and enables a more detailed analysis than previously possible. We discuss 1) the integration of data clustering and visualization into one framework, 2) the application of data clustering to 3D gene expression data, 3) the evaluation of the number of clusters k in the context of 3D gene expression clustering, and 4) the improvement of overall analysis quality via dedicated postprocessing of clustering results based on visualization. We discuss the use of this framework to objectively define spatial pattern boundaries and temporal profiles of genes and to analyze how mRNA patterns are controlled by their regulatory transcription factors.

Inman JL, Bissell MJ. Apical polarity in three-dimensional culture systems: where to now? *Journal of Biology*, 2010 Jan 21;9(1):2. PMID: 20092610

Delineation of the mechanisms that establish and maintain the polarity of epithelial tissues is essential to understanding morphogenesis, tissue specificity and cancer. Three-dimensional culture assays provide a useful platform for dissecting these processes but, as discussed in a recent study in *BMC Biology* on the

culture of mammary gland epithelial cells, multiple parameters that influence the model must be taken into account.

Lavretsky H, Zheng L, Weiner MW, Mungas D, Reed B, Kramer JH, **Jagust W**, Chui H, Mack WJ. Association of depressed mood and mortality in older adults with and without cognitive impairment in a prospective naturalistic study. *The American Journal of Psychiatry*, 2010 Feb 16. [Epub ahead of print]PMID: 20160005

Objective: The authors examined predictors of mortality in individuals age 50 or older with or without cognitive impairment in a 12-year prospective naturalistic study of subcortical ischemic vascular disease focusing on symptoms of depressed mood, apathy, anhedonia, or anergia. Method A total of 498 participants were recruited from the community and from memory clinics into a multicenter longitudinal study of subcortical ischemic vascular disease. For baseline cognitive status, 36% of participants were assessed as cognitively intact, 31% as cognitively impaired, and 33% as demented. All participants underwent a research protocol MRI, and 41% were classified as having subcortical lacunes. Depressed mood, anhedonia, anergia, and apathy were assessed at baseline using a structured behavioral assessment. Cox regression models were used to investigate the associations between neuropsychiatric symptoms and mortality, controlling for age, gender, race, education level, cognitive status, presence of vascular lacunes, and vascular risk factors. Results Of 498 participants, 175 (35%) died over the follow-up period, with a median survival time of 5.6 years. In the multivariate analyses, cognitive impairment, age, male gender, depressed mood, and the presence of lacunes predicted higher mortality. Participants with both lacunes and depressed mood had the shortest survival among all cognitive groups. The mortality hazard ratio for participants with depressed mood was 2.2 (95% CI=1.5-3.2) after adjustment for cognitive status, age, gender, education level, race, lacunes, and all vascular conditions. Conclusions: These findings suggest the importance of detecting depressed mood in individuals with cerebrovascular disease and of developing more aggressive treatment and preventive interventions for this vulnerable population.

Jack CR Jr, Knopman DS, **Jagust WJ**, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurology*, 2010 Jan;9(1):119-28. PMID: 20083042

Currently available evidence strongly supports the position that the initiating event in Alzheimer's disease (AD) is related to abnormal processing of beta-amyloid (Abeta) peptide, ultimately leading to formation of Abeta plaques in the brain. This process occurs while individuals are still cognitively normal. Biomarkers of brain beta-amyloidosis are reductions in CSF Abeta(42) and increased amyloid PET tracer retention. After a lag period, which varies from patient to patient, neuronal dysfunction and neurodegeneration become the dominant pathological processes. Biomarkers of neuronal injury and neurodegeneration are increased CSF tau and structural MRI measures of cerebral atrophy. Neurodegeneration is accompanied by synaptic dysfunction, which is indicated by decreased fluorodeoxyglucose uptake on PET. We propose a model that relates disease stage to AD biomarkers in which Abeta biomarkers become abnormal first, before neurodegenerative biomarkers and cognitive symptoms, and neurodegenerative biomarkers become abnormal later, and correlate with clinical symptom severity.

Korkola J, Gray JW. Breast cancer genomes-form and function. *Current Opinion in Genetics & Development*, 2010 Feb;20(1):4-14. PMID: 20060285

This review summarizes advances in our understanding of the genomic and epigenomic abnormalities in breast cancers that are being revealed by the increasingly powerful suite of genomic analysis technologies. It summarizes the remarkable genomic heterogeneity that characterizes the disease, describes mechanisms that shape cancer genomes as they evolve toward metastasis, summarizes

important recurrent aberrations that exist in spite of the genomic chaos and that contribute to breast cancer pathophysiology, and describes the use of preclinical models to identify drugs that will be effective against subsets of breast cancers carrying specific genomic and epigenomic abnormalities.

Zafar F, Seidler SB, **Kronenberg A, Schild D, Wiese C**. Homologous recombination contributes to the repair of DNA double-strand breaks induced by high-energy iron ions. *Radiation research*, 2010 Jan;173(1):27-39. PMID: 20041757

To test the contribution of homologous recombinational repair (HRR) in repairing DNA damage sites induced by high-energy iron ions, we used (1) HRR-deficient rodent cells carrying a deletion in the RAD51D gene and (2) syngeneic human cells impaired for HRR by RAD51D or RAD51 knockdown using RNA interference. We found that in response to exposure to iron ions, HRR contributed to cell survival in rodent cells and that HRR deficiency abrogated RAD51 focus formation. Complementation of the HRR defect by human RAD51D rescues both enhanced cytotoxicity and RAD51 focus formation. For human cells irradiated with iron ions, cell survival was decreased, and in p53 mutant cells, the levels of mutagenesis were increased when HRR was impaired. Human cells synchronized in S phase exhibited a more pronounced resistance to iron ions compared with cells in G(1) phase, and this increase in radioresistance was diminished by RAD51 knockdown. These results indicate a role for RAD51-mediated DNA repair (i.e. HRR) in removing a fraction of clustered lesions induced by charged-particle radiation. Our results are the first to directly show the requirement for an intact HRR pathway in human cells in ensuring DNA repair and cell survival after exposure to high-energy high-LET radiation.

Kuo WL, Das D, Ziyad S, Bhattacharya S, Gibb WJ, Heiser LM, Sadanandam A, Fontenay GV, Hu Z, Wang NJ, Bayani N, Feiler HS, Neve RM, Wyrobek AJ, Spellman PT, Marton LJ, Gray JW. A systems analysis of the chemosensitivity of breast cancer cells to the polyamine analogue PG-11047. *BMC Medicine*, 2009 Dec 14;7:77. PMID: 20003408

BACKGROUND: Polyamines regulate important cellular functions and polyamine dysregulation frequently occurs in cancer. The objective of this study was to use a systems approach to study the relative effects of PG-11047, a polyamine analogue, across breast cancer cells derived from different patients and to identify genetic markers associated with differential cytotoxicity. METHODS: A panel of 48 breast cell lines that mirror many transcriptional and genomic features present in primary human breast tumours were used to study the antiproliferative activity of PG-11047. Sensitive cell lines were further examined for cell cycle distribution and apoptotic response. Cell line responses, quantified by the GI50 (dose required for 50% relative growth inhibition) were correlated with the omic profiles of the cell lines to identify markers that predict response and cellular functions associated with drug sensitivity. RESULTS: The concentrations of PG-11047 needed to inhibit growth of members of the panel of breast cell lines varied over a wide range, with basal-like cell lines being inhibited at lower concentrations than the luminal cell lines. Sensitive cell lines showed a significant decrease in S phase fraction at doses that produced little apoptosis. Correlation of the GI50 values with the omic profiles of the cell lines identified genomic, transcriptional and proteomic variables associated with response. CONCLUSIONS: A 13-gene transcriptional marker set was developed as a predictor of response to PG-11047 that warrants clinical evaluation. Analyses of the pathways, networks and genes associated with response to PG-11047 suggest that response may be influenced by interferon signalling and differential inhibition of aspects of motility and epithelial to mesenchymal transition.

Purcell JW, Davis J, Reddy M, Martin S, Samayoa K, Vo H, Thomsen K, Bean P, **Kuo WL, Ziyad S, Billig J, Feiler HS, Gray JW, Wood KW, Cases S**. Activity of the kinesin spindle protein inhibitor ispinesib (SB-715992) in models of breast cancer. *Clinical Cancer Research*, 2010 Jan 15;16(2):566-76. Epub 2010 Jan 12. PMID: 20068098

PURPOSE: Ispinesib (SB-715992) is a potent inhibitor of kinesin spindle protein, a kinesin motor protein essential for the formation of a bipolar mitotic spindle and cell cycle progression through mitosis. Clinical studies of ispinesib have shown a 9% response rate in patients with locally advanced or metastatic breast cancer and a favorable safety profile without significant neurotoxicities, gastrointestinal toxicities, or hair loss. To better understand the potential of ispinesib in the treatment of breast cancer, we explored the activity of ispinesib alone and in combination with several therapies approved for the treatment of breast cancer. **EXPERIMENTAL DESIGN:** We measured the ispinesib sensitivity and pharmacodynamic response of breast cancer cell lines representative of various subtypes in vitro and as xenografts in vivo and tested the ability of ispinesib to enhance the antitumor activity of approved therapies. **RESULTS:** In vitro, ispinesib displayed broad antiproliferative activity against a panel of 53 breast cell lines. In vivo, ispinesib produced regressions in each of five breast cancer models and tumor-free survivors in three of these models. The effects of ispinesib treatment on pharmacodynamic markers of mitosis and apoptosis were examined in vitro and in vivo, revealing a greater increase in both mitotic and apoptotic markers in the MDA-MB-468 model than in the less sensitive BT-474 model. In vivo, ispinesib enhanced the antitumor activity of trastuzumab, lapatinib, doxorubicin, and capecitabine and exhibited activity comparable with paclitaxel and ixabepilone. **CONCLUSIONS:** These findings support further clinical exploration of kinesin spindle protein inhibitors for the treatment of breast cancer.

Salisbury Palomares KT, Gerstenfeld LC, Wigner NA, **Lenburg ME**, Einhorn TA, Morgan EF. Transcriptional profiling and biochemical analysis of mechanically induced cartilaginous tissues. *Arthritis and Rheumatism*, 2010 Jan 28. [Epub ahead of print]PMID: 20131271

OBJECTIVE: In order to characterize patterns of molecular expression that lead to cartilage formation in vivo in a post-natal setting, mRNA expression profiling was carried out across the timecourse of mechanically induced chondrogenesis. **METHODS:** Retired breeder Sprague-Dawley rats underwent production of a non-critical-size, transverse femoral osteotomy. Experimental animals (n=45) were subjected to bending stimulation (60 degrees cyclic motion in the sagittal plane for 15 minutes/day) of the osteotomy gap beginning on post-operative day (POD) 10. Control animals (n=32) experienced continuous rigid fixation. mRNA isolated on POD 10, 17, 24, and 38 was analyzed using a microarray containing 608 genes involved in skeletal development, tissue differentiation, fracture healing, and mechanotransduction. The glycosaminoglycan (GAG) content of the stimulated tissues was compared to native articular cartilage as a means of assessing the progression of chondrogenic development of the tissues. **RESULTS:** The majority of the 100 genes that were differentially expressed were upregulated in response to mechanical stimulation. Many of these genes are associated with articular cartilage development and maintenance, diarthroidal joint development, cell adhesion, extracellular matrix synthesis, signal transduction, and skeletal development. Quantitative real-time PCR results were consistent with the microarray findings. The GAG content of the stimulated tissues increased over time and was no different from that of articular cartilage at POD 38. **CONCLUSIONS:** The mechanical stimulation caused upregulation of genes principally involved in joint cavity morphogenesis and critical to articular cartilage function. Further study of this type of stimulation may identify key signaling events required for post-natal, hyaline cartilage formation.

Papageorgis P, Lambert AW, Ozturk S, Gao F, Pan H, Manne U, Alekseyev YO, Thiagalingam A, Abdolmaleky HM, **Lenburg M**, Thiagalingam S. Smad signaling is required to maintain epigenetic silencing during breast cancer progression. *Cancer Research*, 2010 Feb 1;70(3):968-78. PMID: 20086175

Breast cancer progression is associated with aberrant DNA methylation and expression of genes that control the epithelial-mesenchymal transition (EMT), a critical step in malignant conversion. Although the genes affected have been studied, there is little understanding of how aberrant activation of the DNA

methylation machinery itself occurs. Using a breast cancer cell-based model system, we found that cells that underwent EMT exhibited overactive transforming growth factor beta (TGFbeta) signaling and loss of expression of the CDH1, CGN, CLDN4, and KLK10 genes as a result of hypermethylation of their corresponding promoter regions. Based on these observations, we hypothesized that activated TGFbeta-Smad signaling provides an "epigenetic memory" to maintain silencing of critical genes. In support of this hypothesis, disrupting Smad signaling in mesenchymal breast cancer cells resulted in DNA demethylation and reexpression of the genes identified. This epigenetic reversal was accompanied by an acquisition of epithelial morphology and a suppression of invasive properties. Notably, disrupting TGFbeta signaling decreased the DNA binding activity of DNA methyltransferase DNMT1, suggesting that failure to maintain methylation of newly synthesized DNA was the likely cause of DNA demethylation. Together, our findings reveal a hyperactive TGFbeta-TGFbetaR-Smad2 signaling axis needed to maintain epigenetic silencing of critical EMT genes and breast cancer progression.

Mi H, Dong Q, Muruganujan A, Gaudet P, **Lewis S**, Thomas PD. PANTHER version 7: improved phylogenetic trees, orthologs and collaboration with the Gene Ontology Consortium. *Nucleic Acids Research*, 2010 Jan;38(Database issue):D204-10. PMID: 20015972

Protein Analysis Through Evolutionary Relationships (PANTHER) is a comprehensive software system for inferring the functions of genes based on their evolutionary relationships. Phylogenetic trees of gene families form the basis for PANTHER and these trees are annotated with ontology terms describing the evolution of gene function from ancestral to modern day genes. One of the main applications of PANTHER is in accurate prediction of the functions of uncharacterized genes, based on their evolutionary relationships to genes with functions known from experiment. The PANTHER website, freely available at <http://www.pantherdb.org>, also includes software tools for analyzing genomic data relative to known and inferred gene functions. Since 2007, there have been several new developments to PANTHER: (i) improved phylogenetic trees, explicitly representing speciation and gene duplication events, (ii) identification of gene orthologs, including least diverged orthologs (best one-to-one pairs), (iii) coverage of more genomes (48 genomes, up to 87% of genes in each genome; see <http://www.pantherdb.org/panther/summaryStats.jsp>), (iv) improved support for alternative database identifiers for genes, proteins and microarray probes and (v) adoption of the SBGN standard for display of biological pathways. In addition, PANTHER trees are being annotated with gene function as part of the Gene Ontology Reference Genome project, resulting in an increasing number of curated functional annotations.

Kuczynski B, Targan E, **Madison C**, Weiner M, Zhang Y, Reed B, Chui HC, **Jagust W**. White matter integrity and cortical metabolic associations in aging and dementia. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, 2010 Jan;6(1):54-62. PMID: 20129319

BACKGROUND: Studies show that white matter hyperintensities, regardless of location, primarily affect frontal lobe metabolism and function. This report investigated how regional white matter integrity (measured as fractional anisotropy [FA]) relates to brain metabolism, to unravel the complex relationship between white matter changes and brain metabolism. **OBJECTIVE:** To elucidate the relationship between white matter integrity and gray matter metabolism using diffusion tensor imaging and fluorodeoxyglucose-positron emission tomography in a cohort of 16 subjects ranging from normal to demented (age, >55 years). **METHODS:** Mean FA values from white matter regions underlying the medial prefrontal, inferior-lateral prefrontal, parietal association, and posterior temporal areas and the corpus callosum were regressed with glucose metabolism (by positron emission tomography), using statistical parametric mapping ($P < 0.005$; voxel cluster, >100). Regional cerebral glucose metabolism was the primary outcome measure. According to our hypothesis, those hypometabolic cortical regions affected by Alzheimer's disease would correlate with a lower FA of associated tracks. **RESULTS:** Our data show inter-

regional positive correlations between FA and gray matter metabolism for the prefrontal cortex, temporal, and parietal regions. Our results suggest that left prefrontal FA is associated with left temporal and parietal metabolism. Further, left posterior temporal FA correlated with left prefrontal metabolism. Finally, bilateral parietal FA correlated with bilateral temporal metabolism. CONCLUSIONS: These regions are associated with cognitive processes affected in Alzheimer's disease and cerebrovascular disease, suggesting a link with white matter degeneration and gray matter hypometabolism. Therefore, cortical function and white matter degeneration are related in aging and dementia. 2010 The Alzheimer's Association.

Marchetti F, Venkatachalam S. The multiple roles of Bub1 in chromosome segregation during mitosis and meiosis. *Cell Cycle*, 2010 Jan 1;9(1):58-63. PMID: 20016277

Aneuploidy, any deviation from an exact multiple of the haploid number of chromosomes, is a common occurrence in cancer and represents the most frequent chromosomal disorder in newborns. Eukaryotes have evolved mechanisms to assure the fidelity of chromosome segregation during cell division that include a multiplicity of checks and controls. One of the main cell division control mechanisms is the spindle assembly checkpoint (SAC) that monitors the proper attachment of chromosomes to spindle fibers and prevents anaphase until all kinetochores are properly attached. The mammalian SAC is composed of at least 14 evolutionary-conserved proteins that work in a coordinated fashion to monitor the establishment of amphitelic attachment of all chromosomes before allowing cell division to occur. Among the SAC proteins, the budding uninhibited by benzimidazole protein 1 (Bub1), is a highly conserved protein of prominent importance for the proper functioning of the SAC. Studies have revealed many roles for Bub1 in both mitosis and meiosis, including the localization of other SAC proteins to the kinetochore, SAC signaling, metaphase congression and the protection of sister chromatid cohesion. Recent data show striking sex specific differences in the response of germ cells to alterations in Bub1 activity. Proper Bub1 functioning is particularly important during oogenesis in preventing the generation of aneuploid gametes that can have detrimental effects on the health status of the fetus and the newborn. These data suggest that Bub1 is a master regulator of SAC and chromosomal segregation in both mitosis and meiosis. Elucidating its many essential functions in regulating proper chromosome segregation can have important consequences for preventing tumorigenesis and developmental abnormalities.

Ghajar CM, Kachgal S, Kniazeva E, **Mori H, Costes SV**, George SC, Putnam AJ. Mesenchymal cells stimulate capillary morphogenesis via distinct proteolytic mechanisms. *Experimental Cell Research*, 2010 Jan 11. [Epub ahead of print] PMID: 20067788

During angiogenesis, endothelial cells (ECs) degrade their surrounding extracellular matrix (ECM) to facilitate invasion. How interactions between ECs and other cells within their microenvironment facilitate this process is only partially understood. We have utilized a tractable 3D co-culture model to investigate the proteolytic mechanisms by which pre-committed or more highly committed mesenchymal cells stimulate capillary formation. On their own, ECs invade their surrounding matrix, but do not form capillaries. However, in the presence of either mesenchymal stem cells (MSCs) or fibroblasts, ECs form polarized, tubular structures that are intimately associated with mesenchymal cells. Further, ECs up-regulate gene expression of several extracellular proteases upon co-culture with either mesenchymal cell type. The administration of both broad spectrum and specific protease inhibitors demonstrated that MSC-stimulated capillary formation relied solely on membrane-type matrix metalloproteinases (MT-MMPs) while fibroblast-mediated sprouting proceeded independent of MMP inhibition unless the plasminogen activator/plasmin axis was inhibited in concert. While other studies have established a role for the ECM itself in dictating proteolysis and matrix degradation during capillary morphogenesis, the present study illustrates that heterotypic cellular interactions within the microenvironment can direct the proteolytic mechanisms required for capillary formation.

Xie QG, Kao CM, Wang X, Guo N, Frisch H, **Moses WW**, Chen CT. Potentials of digitally sampling scintillation pulses in timing determination in PET. *IEEE Transactions on Nuclear Science*, 2009 Oct; 56(5):2607-2613.

We investigate the potentials of digitally sampling scintillation pulses techniques for positron emission tomography (PET) in this paper, focusing on the determination of the event time. We have built, and continue building, a digital library of PET event waveforms generated with various combinations of photo-detectors and scintillator materials, with various crystal sizes. Events in this digital library are obtained at a high sampling of 20 GSps (Giga-samples per second) so that their waveforms are recorded with high accuracy. To explore the potential advantages of digitally sampling scintillation pulses, we employ a dataset in the above-mentioned library to evaluate two methods for digitizing the event pulses and linear interpolation techniques to analyze the resulting digital samples. Our results show that the two digitization methods that we studied can yield a coincidence timing resolution of about 300 ps FWHM when applied to events generated by a pair of LSO + PMT detector units. This timing resolution is comparable with that is achieved by the same detector pair with a constant fraction discriminator (CFD). As a benchmark, regular-time sampling (RTS) method, usually implemented with very fast traditional analog-to-digital converters (ADCs) for digitizing scintillation pulses, is not feasible for a multi-channel system like a PET system. Digitizing scintillation pulses with multi-voltage threshold (MVT) method could be implemented at a reasonable cost for a PET system. With digitized PET event samples, various digital signal processing (DSP) techniques can be implemented to determine event arrival time. Our results have therefore demonstrated the promising potentials of digitally sampling scintillation pulses techniques in PET imaging.

Mukhopadhyay R, Costes S, Bazarov A, Hines WC, Barcellos-Hoff MH, Yaswen P. Promotion of variant human mammary epithelial cell outgrowth by ionizing radiation: an agent-based model supported by in vitro studies. *Breast Cancer Research*, 2010 Feb 10;12(1):R11. PMID: 20146798

INTRODUCTION: Most human mammary epithelial cells (HMEC) cultured from histologically normal breast tissues enter a senescent state termed stasis after 5-20 population doublings. These senescent cells display increased size, contain senescence associated beta-galactosidase activity, and express cyclin-dependent kinase inhibitor, p16INK4A (CDKN2A; p16). However, HMEC grown in a serum-free medium, spontaneously yield, at low frequency, variant (v) HMEC that are capable of long-term growth and are susceptible to genomic instability. We investigated whether ionizing radiation, which increases breast cancer risk in women, affects the rate of vHMEC outgrowth. METHODS: Pre-stasis HMEC cultures were exposed to 5-200 cGy of sparsely (X- or gamma-rays) or densely (1 GeV/amu 56Fe) ionizing radiation. Proliferation (bromodeoxyuridine incorporation), senescence (senescence-associated beta-galactosidase activity), and p16 expression were assayed in subcultured irradiated or unirradiated populations 4-6 weeks following radiation exposure, when patches of vHMEC became apparent. Long-term growth potential and p16 promoter methylation in subsequent passages were also monitored. Agent-based modeling, incorporating a simple set of rules and underlying assumptions, was used to simulate vHMEC outgrowth and evaluate mechanistic hypotheses. RESULTS: Cultures derived from irradiated cells contained significantly more vHMEC, lacking senescence associated beta-galactosidase or p16 expression, than cultures derived from unirradiated cells. As expected, post-stasis vHMEC cultures derived from both unirradiated and irradiated cells exhibited more extensive methylation of the p16 gene than pre-stasis HMEC cultures. However, the extent of methylation of individual CpG sites in vHMEC samples did not correlate with passage number or treatment. Exposure to sparsely or densely ionizing radiation elicited similar increases in the numbers of vHMEC compared to unirradiated controls. Agent-based modeling indicated that radiation-induced premature senescence of normal HMEC most likely accelerated vHMEC outgrowth through alleviation of spatial constraints. Subsequent experiments using defined co-cultures of

vHMEC and senescent cells supported this mechanism. **CONCLUSIONS:** Our studies indicate that ionizing radiation can promote the outgrowth of epigenetically altered cells with pre-malignant potential.

Mungall CJ, Gkoutos GV, Smith CL, Haendel MA, **Lewis SE**, Ashburner M. Integrating phenotype ontologies across multiple species. *Genome Biology*, 2010 Jan 8;11(1):R2. PMID: 20064205

Phenotype ontologies are typically constructed to serve the needs of a particular community, such as annotation of genotype-phenotype associations in mouse or human. Here we demonstrate how these ontologies can be improved through assignment of logical definitions using a core ontology of phenotypic qualities and multiple additional ontologies from the Open Biological Ontologies library. We also show how these logical definitions can be used for data integration when combined with a unified multi-species anatomy ontology.

Shatsky M, Hall RJ, **Nogales E**, Malik J, Brenner SE. Automated multi-model Reconstruction from Single-Particle Electron Microscopy Data. *Journal of Structural Biology*, 2010 Jan 16. [Epub ahead of print] PMID: 20085819

Biological macromolecules can adopt multiple conformational and compositional states due to structural flexibility and alternative subunit assemblies. This structural heterogeneity poses a major challenge in the study of macromolecular structure using single particle electron microscopy. We propose a fully automated, unsupervised method for the three-dimensional reconstruction of multiple structural models from heterogeneous data. As a starting reference, our method employs an initial structure that does not account for any heterogeneity. Then, a multi-stage clustering is used to create multiple models representative of the heterogeneity within the sample. The multi-stage clustering combines an existing approach based on Multivariate Statistical Analysis to perform clustering within individual Euler angles, and a newly developed approach to sort out class-averages from individual Euler angles into homogeneous groups. Structural models are computed from individual clusters. The whole data classification is further refined using an iterative multi-model projection matching approach. We tested our method on one synthetic and three distinct experimental datasets. The tests include the cases where a macromolecular complex exhibits structural flexibility and cases where a molecule is found in ligand-bound and unbound states. We propose the use of our approach as an efficient way to reconstruct distinct multiple models from heterogeneous data.

Verhaak RG, Hoadley KA, **Purdom E**, **Wang V**, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, **Jakkula L**, **Feiler HS**, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, **Spellman PT**, Wilson RK, Speed TP, **Gray JW**, Meyerson M, Getz G, Perou CM, Hayes DN; Cancer Genome Atlas Research Network. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*, 2010 Jan 19;17(1):98-110. PMID: 20129251

The Cancer Genome Atlas Network recently cataloged recurrent genomic abnormalities in glioblastoma multiforme (GBM). We describe a robust gene expression-based molecular classification of GBM into Proneural, Neural, Classical, and Mesenchymal subtypes and integrate multidimensional genomic data to establish patterns of somatic mutations and DNA copy number. Aberrations and gene expression of EGFR, NF1, and PDGFRA/IDH1 each define the Classical, Mesenchymal, and Proneural subtypes, respectively. Gene signatures of normal brain cell types show a strong relationship between subtypes and different neural lineages. Additionally, response to aggressive therapy differs by subtype, with the greatest benefit in the Classical subtype and no benefit in the Proneural subtype. We provide a framework that unifies transcriptomic and genomic dimensions for GBM molecular stratification with important implications for future studies. Copyright (c) 2010 Elsevier Inc. All rights reserved.

Rabinovici GD, Furst AJ, Alkalay A, Racine CA, O'Neil JP, Janabi M, Baker SL, Agarwal N, Bonasera SJ, Mormino EC, Weiner MW, Gorno-Tempini ML, Rosen HJ, Miller BL, Jagust WJ. Increased metabolic vulnerability in early-onset Alzheimer's disease is not related to amyloid burden. *Brain: A Journal of Neurology*, 2010 Feb;133(Pt 2):512-28. PMID: 20080878

Patients with early age-of-onset Alzheimer's disease show more rapid progression, more generalized cognitive deficits and greater cortical atrophy and hypometabolism compared to late-onset patients at a similar disease stage. The biological mechanisms that underlie these differences are not well understood. The purpose of this study was to examine in vivo whether metabolic differences between early-onset and late-onset Alzheimer's disease are associated with differences in the distribution and burden of fibrillar amyloid-beta. Patients meeting criteria for probable Alzheimer's disease (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's; Disease and Related Disorders Association criteria) were divided based on estimated age at first symptom (less than or greater than 65 years) into early-onset (n = 21, mean age-at-onset 55.2 +/- 5.9 years) and late-onset (n = 18, 72.0 +/- 4.7 years) groups matched for disease duration and severity. Patients underwent positron emission tomography with the amyloid-beta-ligand [(11)C]-labelled Pittsburgh compound-B and the glucose analogue [(18)F]-labelled fluorodeoxyglucose. A group of cognitively normal controls (n = 30, mean age 73.7 +/- 6.4) was studied for comparison. [(11)C]-labelled Pittsburgh compound-B images were analysed using Logan graphical analysis (cerebellar reference) and [(18)F]-labelled fluorodeoxyglucose images were normalized to mean activity in the pons. Group differences in tracer uptake were assessed on a voxel-wise basis using statistical parametric mapping, and by comparing mean values in regions of interest. To account for brain atrophy, analyses were repeated after applying partial volume correction to positron emission tomography data. Compared to normal controls, both early-onset and late-onset Alzheimer's disease patient groups showed increased [(11)C]-labelled Pittsburgh compound-B uptake throughout frontal, parietal and lateral temporal cortices and striatum on voxel-wise and region of interest comparisons (P < 0.05). However, there were no significant differences in regional or global [(11)C]-labelled Pittsburgh compound-B binding between early-onset and late-onset patients. In contrast, early-onset patients showed significantly lower glucose metabolism than late-onset patients in precuneus/posterior cingulate, lateral temporo-parietal and occipital cortices (voxel-wise and region of interest comparisons, P < 0.05). Similar results were found for [(11)C]-labelled Pittsburgh compound-B and [(18)F]-labelled fluorodeoxyglucose using atrophy-corrected data. Age-at-onset correlated positively with glucose metabolism in precuneus, lateral parietal and occipital regions of interest (controlling for age, education and Mini Mental State Exam, P < 0.05), while no correlations were found between age-at-onset and [(11)C]-labelled Pittsburgh compound-B binding. In summary, a comparable burden of fibrillar amyloid-beta was associated with greater posterior cortical hypometabolism in early-onset Alzheimer's disease. Our data are consistent with a model in which both early amyloid-beta accumulation and increased vulnerability to amyloid-beta pathology play critical roles in the pathogenesis of Alzheimer's disease in young patients

Nishimura N, Hitomi K, Arvai AS, **Rambo RP**, Hitomi C, Cutler SR, Schroeder JI, Getzoff ED. Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. *Science*, 2009 Dec 4;326(5958):1373-9.

See also News Highlight, page 5.

The phytohormone abscisic acid (ABA) acts in seed dormancy, plant development, drought tolerance, and adaptive responses to environmental stresses. Structural mechanisms mediating ABA receptor recognition and signaling remain unknown but are essential for understanding and manipulating abiotic stress resistance. Here, we report structures of pyrabactin resistance 1 (PYR1), a prototypical PYR/PYR1-like (PYL)/regulatory component of ABA receptor (RCAR) protein that functions in early ABA signaling. The

crystallographic structure reveals an alpha/beta helix-grip fold and homodimeric assembly, verified in vivo by coimmunoprecipitation. ABA binding within a large internal cavity switches structural motifs distinguishing ABA-free "open-lid" from ABA-bound "closed-lid" conformations. Small-angle x-ray scattering suggests that ABA signals by converting PYR1 to a more compact, symmetric closed-lid dimer. Site-directed PYR1 mutants designed to disrupt hormone binding lose ABA-triggered interactions with type 2C protein phosphatase partners in planta.

Rambo RP, Tainer JA. Bridging the solution divide: comprehensive structural analyses of dynamic RNA, DNA, and protein assemblies by small-angle X-ray scattering. *Current Opinion in Structural Biology*, 2010 Feb;20(1):128-37. PMID: 20097063

Small-angle X-ray scattering (SAXS) is changing how we perceive biological structures, because it reveals dynamic macromolecular conformations and assemblies in solution. SAXS information captures thermodynamic ensembles, enhances static structures detailed by high-resolution methods, uncovers commonalities among diverse macromolecules, and helps define biological mechanisms. SAXS-based experiments on RNA riboswitches and ribozymes and on DNA-protein complexes including DNA-PK and p53 discover flexibilities that better define structure-function relationships. Furthermore, SAXS results suggest conformational variation is a general functional feature of macromolecules. Thus, accurate structural analyses will require a comprehensive approach that assesses both flexibility, as seen by SAXS, and detail, as determined by X-ray crystallography and NMR. Here, we review recent SAXS computational tools, technologies, and applications to nucleic acids and related structures.

Rambo RP, Tainer JA. Improving small-angle X-ray scattering data for structural analyses of the RNA world. *RNA*, 2010 Jan 27. [Epub ahead of print] PMID: 20106957

Defining the shape, conformation, or assembly state of an RNA in solution often requires multiple investigative tools ranging from nucleotide analog interference mapping to X-ray crystallography. A key addition to this toolbox is small-angle X-ray scattering (SAXS). SAXS provides direct structural information regarding the size, shape, and flexibility of the particle in solution and has proven powerful for analyses of RNA structures with minimal requirements for sample concentration and volumes. In principle, SAXS can provide reliable data on small and large RNA molecules. In practice, SAXS investigations of RNA samples can show inconsistencies that suggest limitations in the SAXS experimental analyses or problems with the samples. Here, we show through investigations on the SAM-I riboswitch, the Group I intron P4-P6 domain, 30S ribosomal subunit from *Sulfolobus solfataricus* (30S), bromo mosaic virus tRNA-like structure (BMV-TLS), *Thermotoga maritima* asd lysine riboswitch, the recombinant tRNA(val), and yeast tRNA(phe) that many problems with SAXS experiments on RNA samples derive from heterogeneity of the folded RNA. Furthermore, we propose and test a general approach to reducing these sample limitations for accurate SAXS analyses of RNA. Together our method and results show that SAXS with synchrotron radiation has great potential to provide accurate RNA shapes, conformations, and assembly states in solution that inform RNA biological functions in fundamental ways.

Spidlen J, Moore W, Parks D, Goldberg M, Bray C, Bierre P, Gorombey P, Hyun B, Hubbard M, Lange S, Lefebvre R, Leif R, Novo D, Ostruszka L, Treister A, Wood J, Murphy RF, Roederer M, **Sudar D**, Zigon R, Brinkman RR. Data file standard for flow cytometry, version FCS 3.1. *Cytometry Part A : The Journal of the International Society for Analytical Cytology*, 2010 Jan;77(1):97-100 PMID: 19937951

The flow cytometry data file standard provides the specifications needed to completely describe flow cytometry data sets within the confines of the file containing the experimental data. In 1984, the first Flow Cytometry Standard format for data files was adopted as FCS 1.0. This standard was modified in 1990 as FCS 2.0 and again in 1997 as FCS 3.0. We report here on the next generation flow cytometry

standard data file format. FCS 3.1 is a minor revision based on suggested improvements from the community. The unchanged goal of the standard is to provide a uniform file format that allows files created by one type of acquisition hardware and software to be analyzed by any other type. The FCS 3.1 standard retains the basic FCS file structure and most features of previous versions of the standard. Changes included in FCS 3.1 address potential ambiguities in the previous versions and provide a more robust standard. The major changes include simplified support for international characters and improved support for storing compensation. The major additions are support for preferred display scale, a standardized way of capturing the sample volume, information about originality of the data file, and support for plate and well identification in high throughput, plate based experiments. Please see the normative version of the FCS 3.1 specification in Supporting Information for this manuscript (or at <http://www.isac-net.org/> in the Current standards section) for a complete list of changes.

Williams JS, Williams RS, Dovey CL, Guenther G, **Tainer JA**, Russell P. gammaH2A binds Brc1 to maintain genome integrity during S-phase. *The EMBO Journal*, 2010 Jan 21. [Epub ahead of print] PMID: 20094029

ATM(Tel1) and ATR(Rad3) checkpoint kinases phosphorylate the C-terminus of histone H2AX (H2A in yeasts) in chromatin flanking DNA damage, establishing a recruitment platform for checkpoint and repair proteins. Phospho-H2A/X (gammaH2A/X)-binding proteins at double-strand breaks (DSBs) have been characterized, but those required for replication stress responses are unknown. Here, we present genetic, biochemical, small angle X-ray scattering (SAXS), and X-ray structural studies of the *Schizosaccharomyces pombe* Brc1, a 6-BRCT-domain protein that is structurally related to *Saccharomyces cerevisiae* Rtt107 and mammalian PTIP. Brc1 binds gammaH2A to form spontaneous and DNA damage-induced nuclear foci. Spontaneous Brc1 foci colocalize with ribosomal DNA repeats, a region prone to fork pausing and genomic instability, whereas DNA damage-induced Brc1 foci colocalize with DSB response factors. gammaH2A binding is critical for Brc1 function. The 1.45 Å resolution crystal structure of Brc1-gammaH2A complex shows how variable BRCT insertion loops sculpt tandem-BRCT phosphoprotein-binding pockets to facilitate unique phosphoprotein-interaction specificities, and unveils an acidic DNA-mimicking Brc1 surface. From these results, Brc1 docking to gammaH2A emerges as a critical chromatin-specific response to replication-associated DNA damage.

Das D, Moiani D, Axelrod HL, Miller MD, McMullan D, Jin KK, Abdubek P, Astakhova T, Burra P, Carlton D, Chiu HJ, Clayton T, Deller MC, Duan L, Ernst D, Feuerhelm J, Grant JC, Grzechnik A, Grzechnik SK, Han GW, Jaroszewski L, Klock HE, Knuth MW, Kozbial P, Krishna SS, Kumar A, Marciano D, Morse AT, Nigoghossian E, Okach L, Paulsen J, Reyes R, Rife CL, Sefcovic N, Tien HJ, Trame CB, van den Bedem H, Weekes D, Xu Q, Hodgson KO, Wooley J, Elsliger MA, Deacon AM, Godzik A, Lesley SA, **Tainer JA**, Wilson IA. Crystal structure of the first eubacterial Mre11 nuclease reveals novel features that might discriminate substrates during DNA repair. *Journal of Molecular Biology*, 2010 Feb 1. [Epub ahead of print] PMID: 20122942

Mre11 nuclease plays a central role in the repair of cytotoxic and mutagenic DNA double-strand breaks. As X-ray structural information has been available only for the *Pyrococcus furiosus* enzyme (PfMre11), the conserved and variable features of this nuclease across the domains of life have not been experimentally defined. Our crystal structure and biochemical studies demonstrate that TM1635 from *Thermotoga maritima*, originally annotated as a putative nuclease, is the Mre11 endo/exonuclease from *T. maritima* (TmMre11) and the first such structure from eubacteria. TmMre11 and PfMre11 display similar overall structures, despite sequence identity in the twilight zone of only approximately 20%. However, they differ substantially in their DNA-specificity domains and in their dimeric organization. Residues in the nuclease domain are highly conserved, but those in the DNA-specificity domain are not. The structural differences likely affect how Mre11 from different organisms recognize and interact with single-stranded DNA, double-stranded DNA and DNA hairpin structures during DNA repair. The TmMre11 nuclease active site

has no bound metal ions, but is conserved in sequence and structure with the exception of a histidine that is important in Pfmre11 nuclease activity. Nevertheless, biochemical characterization confirms that Tmmre11 possesses both endonuclease and exonuclease activities on single-stranded and double-stranded DNA substrates, respectively.

lyer RR, Pluciennik A, Genschel J, **Tsai MS**, Beese LS, Modrich P. MutL{alpha} and proliferating cell nuclear antigen share binding sites on Muts{beta}. *Journal of Biological Chemistry*, 2010 Feb 16. [Epub ahead of print] PMID: 20154325

MutSbeta (MSH2-MSH3) mediates repair of insertion-deletion heterologies but also triggers triplet repeat expansions that cause neurological diseases. Like other DNA metabolic activities, MutSbeta interacts with proliferating cell nuclear antigen (PCNA) via a conserved motif (QxxL/lxxFF). We demonstrate that MutSbeta-PCNA complex formation occurs with an affinity of ~ 0.1 muM, and a preferred stoichiometry of 1:1. However, upto 20% of complexes are multivalent under conditions where MutSbeta is in molar excess over PCNA. Conformational studies indicate that the two proteins associate in an end-to-end fashion in solution. Surprisingly, mutation of the PCNA-binding motif of MutSbeta not only abolishes PCNA binding, but unlike MutSalpha, also dramatically attenuates MutSbeta-MutLalpha interaction, MutLalpha endonuclease activation, and bidirectional mismatch repair. As predicted by these findings, PCNA competes with MutLalpha for binding to MutSbeta, an effect that is blocked by the cell cycle regulator p21(CIP1). We propose that MutSbeta-MutLalpha interaction is mediated in part by residues (L/ISRFF) embedded within the MSH3 PCNA-binding motif. To our knowledge this is the first case where residues important for PCNA-binding also mediate interaction with a second protein. These findings also indicate that MutSbeta and MutSalpha-initiated repair events differ in fundamental ways.

Lu CM, Kwan J, **Weier JF**, Baumgartner A, Wang M, Escudero T, Munné S, **Weier HU**. Rapid mapping of chromosomal breakpoints: from blood to BAC in 20 days. *Folia histochemica et cytobiologica*, 2009 Jan 1;47(3):367-75. PMID: 20164020

Structural chromosome aberrations and associated segmental or chromosomal aneusomies are major causes of reproductive failure in humans. Despite the fact that carriers of reciprocal balanced translocation often have no other clinical symptoms or disease, impaired chromosome homologue pairing in meiosis and karyokinesis errors lead to over-representation of translocation carriers in the infertile population and in recurrent pregnancy loss patients. At present, clinicians have no means to select healthy germ cells or balanced zygotes in vivo, but in vitro fertilization (IVF) followed by preimplantation genetic diagnosis (PGD) offers translocation carriers a chance to select balanced or normal embryos for transfer. Although a combination of telomeric and centromeric probes can differentiate embryos that are unbalanced from normal or unbalanced ones, a seemingly random position of breakpoints in these IVF-patients poses a serious obstacle to differentiating between normal and balanced embryos, which for most translocation couples, is desirable. Using a carrier with reciprocal translocation t(4;13) as an example, we describe our state-of-the-art approach to the preparation of patient-specific DNA probes that span or 'extent' the breakpoints. With the techniques and resources described here, most breakpoints can be accurately mapped in a matter of days using carrier lymphocytes, and a few extra days are allowed for PGD-probe optimization. The optimized probes will then be suitable for interphase cell analysis, a prerequisite for PGD since blastomeres are biopsied from normally growing day 3 - embryos regardless of their position in the mitotic cell cycle. Furthermore, routine application of these rapid methods should make PGD even more affordable for translocation carriers enrolled in IVF programs.