



## LIFE SCIENCES DIVISION E-NEWSLETTER

April, 2009

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## DOE scientific focus area notes

### Low Dose Radiation Research

#### Life Sciences Symposium at DOE Low Dose Program Investigators' Workshop

Several Life Sciences Division researchers participated in the annual Low Dose Radiation Research Program Investigators' Workshop VIII held on April 6-8, 2009, in Bethesda, MD. They represented the Life Sciences Low Dose Radiation Research Program (Low Dose Program), which was constituted under the auspices of the Scientific Focus Area (SFA) funding paradigm of the Department of Energy.

The overall goal of the Program is to provide compelling experimental studies that can be used to guide accurate regulatory standards for exposure to low dose radiation. Program researchers address the cell and molecular biology, human genetics and tissue physiology that determine responses to low-dose radiation. Specifically, they are investigating the impact of low dose radiation on three biological radiation-response processes: (a) adaptive responses, (b) non-targeted responses, and (c) epigenetic regulation in relation to cancer, which is the clearest radiation-induced health effect and the endpoint that can be most easily translated into radiation regulatory models. These research directions are supported by advanced technology and conducted in relevant experimental models.

The following Life Sciences researchers presented a half-day symposium session at the workshop that highlighted their research progress in low dose radiation biology: **Priscilla Cooper** - Low Dose Responses and Adaptive Mechanisms: Role of DNA Repair and p53; **Andrew Wyrobek** - Mouse Models for Low Dose and AR Networks in Tissues; **Judith Campisi** - AR are Senescence; **Eleanor Blakely** - Multicellular Crosstalk in Radiation Damage; **Terumi Kohwi-Shigematsu** - The role of the Epigenome; **Trent Northen** - New approaches to in situ Metabolomics; **Jian-Hua Mao** -- Identification of Genetic Modifiers of Susceptibility to Low Dose Radiation Induced Tumor Genesis; **Joe Gray**-- Life Sciences SFA overview, new directions.

Life Sciences researchers also made 15 poster presentations. More information about the workshop, including the poster presentations and complete agenda, can be found at <http://www.orau.gov/lowdose2009/default.htm> . The workshop was sponsored by the U.S. Department of Energy, Office of Biological and Environmental Research.

*Andrew Wyrobek, 4/09*

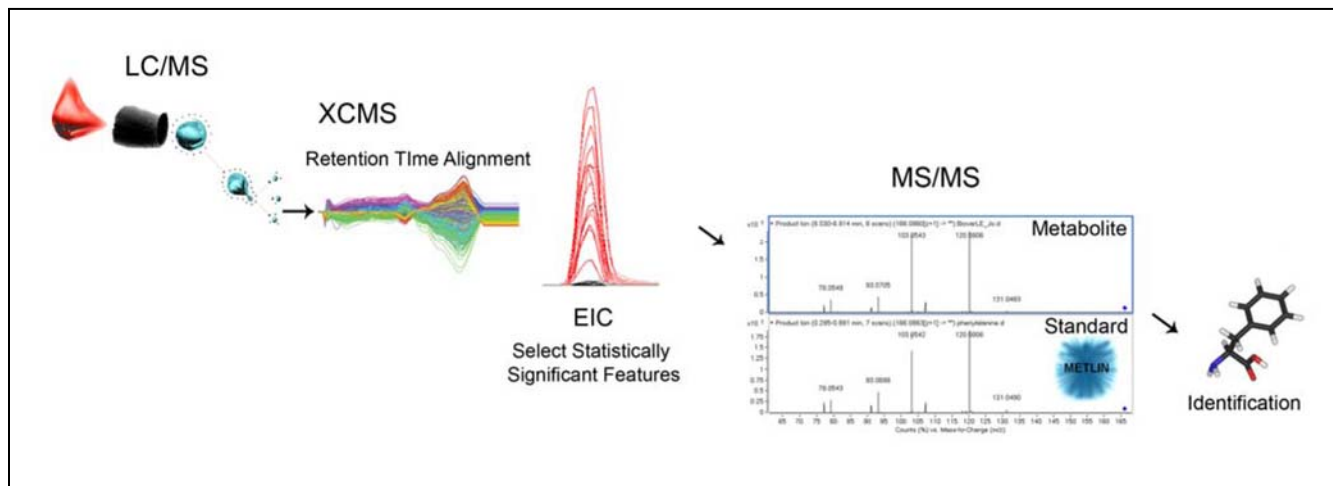
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### GTL-Genomics

#### News from the Emerging Field of Metabolomics

As part of the MAGGIE – Berkeley Lab effort, the **Siuzdak lab** has focused on the emerging field of metabolomics, involving small endogenous metabolites which are ubiquitous in living systems. Three recent developments from the lab include a bioinformatics platform, the identification of novel functionally active metabolites, and a new mass spectrometry technology. For informatics, the lab has developed XCMS and XCMS2 (available open source software) which statistically evaluate LC/MS metabolite features and also uses their metabolite (METLIN) database to structurally characterize them

(Benton HP, Wong, DM, Trauger SA, Siuzdak G. XCMS2: processing tandem mass spectrometry data for metabolite identification and structural characterization. *Analytical Chemistry*, 2008, 80(16), 6382-9. PMID: 18627180). This software originally was the most highly cited paper in *Analytical Chemistry* and in its new form it is helpful with identifying important metabolites for MAGGIE.



In a MAGGIE applied metabolomics study, polyamines were identified to be strongly regulated by an environmental adaptation (Trauger SA, Kalisak E, Kalisiak J, Morita H, Weinberg MV, Menon AL, Poole FL 2nd, Adams MW, Siuzdak G. Correlating the transcriptome, proteome, and metabolome in the environmental adaptation of a hyperthermophile. *J. Proteome Research*, 2008 Mar;7(3):1027-35. PMID: 18247545). One of these was identified as a novel branched structure of N4-(N-acetylaminopropyl) spermidine through an iterative process of chemical synthesis and tandem MS based characterization using accurate mass (Kalisiak J, Trauger SA, Kalisiak E, Morita H, Fokin VV, Adams MW, Sharpless KB, Siuzdak G. Identification of a new endogenous metabolite and the characterization of its protein interactions through an immobilization approach. *Journal American Chemistry Society*, 2009 Jan 14;131(1):378-86. PMID: 19055353). Since this metabolite represented a completely new molecule for which the biosynthetic pathways are unknown, an immobilization and proteomics approach was devised to help identify proteins which interact strongly with it. This approach provides a first glimpse into the probable role of novel metabolites in MAGGIE organisms.

The Siuzdak lab has also developed a new analytical technology - Nanostructure Initiator Mass Spectrometry (NIMS) for analyzing living organisms. NIMS is a recently introduced highly sensitive platform that requires minimal sample preparation. This technique was applied towards single cell analysis as well as enzyme activity. (Yanes O, Woo HK, Northen TR, Oppenheimer SR, Shriver L, Apon J, Estrada MN, Potchoiba MJ, Steenwyk R, Manchester M, Siuzdak G. *Analytical Chemistry*, 2009 Apr 15;81(8):2969-75. PMID: 19301920 & Northen TR, Yanes O, Northen MT, Marrinucci D, Uritboonthai W, Apon J, Golledge SL, Nordstrom A, Siuzdak G. Clathrate nanostructures for mass spectrometry *Nature*, 2007, 449(7165), 1033-U3).  
 Gary Siuzdak, 4/09

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## Nuclear Medicine

### **Budinger to Give Tribute to Anger at Nuclear Medicine Meeting**

**Thomas Budinger** will give the invited lecture at the Society of Nuclear Medicine's 56th Annual Meeting, June 13–17, 2009, in Toronto, Ontario, Canada. Budinger's lecture will be a tribute to Hal O. Anger, who has had a tremendous influence on medical research and diagnostics with his invention of a gamma-based scintillation scanning camera known as the Anger camera. Anger came to Berkeley Lab following World War II and worked there until his retirement in 1982, primarily developing imaging technologies. The Anger camera, which enables physicians to track metabolic processes in the human body, is still in wide use today. He also developed the well counter, widely used in laboratory tests with small samples of radioactive materials.

*Stephen Derenzo, 4/09*

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### **"High-Throughput Discovery of Scintillation Materials" Project Presented**

Life Sciences **Stephen Derenzo** and Edith Bourret-Courchesne, of the Materials Sciences Division, attended the second Department of Homeland Security Academic Research Initiative Grantees Conference in Washington, D.C., April 6–9, 2009. On the first day, Derenzo gave an invited presentation on the Berkeley Lab "High-Throughput Discovery of Scintillation Materials" project, and researchers from other National Labs and private corporations gave presentations on detector materials, detector systems and nuclear threat recognition software projects. For the remaining two and one-half days, researchers from universities across the nation gave presentations on their projects in these areas. Administrators from the Department of Homeland Security also gave overview presentations on the scope of their efforts in detecting nuclear threats across the globe and the need for improved nuclear radiation detector systems.

*Stephen Derenzo, 4/09*

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## Scientific & divisional news

### **Research Supports Existence of Alternative pre-mRNA Splicing Program**

Alternative pre-mRNA splicing permits a single gene to encode multiple protein isoforms that may differ in structure and function. Recent studies suggest that many alternative splicing events are programmed in tissue-specific fashion during normal development and differentiation. With colleagues at Affymetrix and the University of Chicago, **John Conboy's** lab analyzed stage-specific changes in the human late erythroid transcriptome via use of high-resolution microarrays that detect altered expression of individual exons. Ten robust "switches" in exon splicing patterns were identified by microarray analysis and confirmed by RT-PCR, predicting changes in erythroid structural proteins and RNA processing factors. Sequence analysis of the affected exons suggests that the splicing switches represent an active regulatory process rather than a loss of splicing fidelity. Together, these results support the existence of a regulated alternative pre-mRNA splicing program that is critical for synthesis of the appropriate stage-specific proteome as erythroid cells become progressively more specialized during terminal differentiation.

Yamamoto ML, Clark TA, Gee SL, Kang JA, Schweitzer AC, Wickrema A, Conboy JG. Alternative pre-mRNA splicing switches modulate gene expression in late erythropoiesis. *Blood*. 2009 Apr 2;113(14):3363-70. [Epub ahead of print: Feb 4.] PMID: 19196664

*John Conboy, 4/09*

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### **Life Sciences Scientists Present at 100<sup>th</sup> AACR Annual Meeting**

April 18-22, 2009 marked the 100<sup>th</sup> Annual Meeting for the American Association for Cancer Research (AACR). Several Life Sciences scientists participated in this meeting, held in Denver, CO. **Joe Gray** presented in a session entitled, HER2- Targeting Therapies, Resistance, and Counter-acting Strategies with a talk entitled “Novel and not so novel biomarkers of response and resistance to inhibitors of HER2 (ErbB2)”. Additionally, Gray moderated a forum, “Cancer Systems Biology: Fact or Fiction?”, with Peter Sorger and Allan Balmain speaking and he spoke at the 2009 Scientist Survivor’s Program. As chairman of the AACR Pezcoller Foundation Award Committee, Gray also introduced this year's Pezcoller award winner, Napoleone Ferrara of Genetech, Inc.

At the opening Plenary Session, **Mina J. Bissell** presented a talk entitled “The importance of the tumor microenvironment in the initiation, promotion, and therapy of cancer.” **Judith Campisi** spoke at the Cell Cycle and Senescence in Cancer Symposia. Her presentation, “The senescence-associated secretory phenotype,” was one of four presentations in the Symposia. The session covered recent developments in understanding the molecular events that control normal cell cycle progression and senescence; new data on the pathways that control progress through mitosis, and novel insights into the function of the p27 cell cycle regulator; surprising deleterious effects of senescence – for example in aging – and the senescence-associated secretion of proteins that can have a profound effect on promoting the aggressiveness of neighboring cancer cells; lastly the session discussed role of the p53 tumor suppressor protein in controlling cell cycle progression, senescence and cell survival. Campisi also participated in the Women in Cancer Research mentoring session, Roundtable Topic, “Making the Transition to Independence.”

**Zhi Hu**, a Postdoctoral Fellow of the Gray lab, gave a poster presentation. Hu commented on her experience, “I had very good interactions with visitors of academic and industry parties from different places such as MD Anderson Cancer Center, City of Hope at L.A, UK, Italy and Japan. People were very impressed with our strategies on identification of responsive biomarkers of small molecule inhibitor of CENPE.” Likewise, **Angujar Sadanandam**, also of the Gray Lab, presented a poster titled “Integrated omic analysis of breast cancer cell lines.”

80 hours of selected Annual Meeting talks, including talks of Joe Gray and Mina Bissell, are available as free online webcasts here: <http://www.aacr.org/home/scientists/meetings--workshops/aacr-100th-annual-meeting-2009/webcasts.aspx>.

*Judith Novak, 4/09*

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### Moses to Serve on NIH Study Section

**William Moses** will serve as a member of the Biomedical Imaging Technology Study Section, Center for Scientific Review, National Institutes of Health, for the term beginning July 1, 2009 and ending June 30, 2013.

*Stephen Derenzo, 4/09*

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### Taylor Honored for Assistance with HSS Review

Aundra Richards, head of the Department of Energy Site Office at Berkeley Lab (BSO), hosted a special event on April 13, 2009 to honor the excellent work of staff who helped with the Health, Safety and Security (HSS) Review that took place earlier this year. Among those honored was Life Sciences Safety Officer **Scott Taylor**. Richards lauded the group for “their support of the review, which resulted in significant improvements to the implementation of Integrated Safety Management at Berkeley Lab,” and for their collaboration with BSO, which “helped ensure a positive outcome.” Those honored received certificates of appreciation and were treated to refreshments.

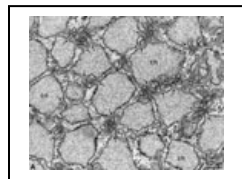
*Today at Berkeley Lab, CG, 4/14/09*



Scott Taylor among others honored

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### The Membrane Metro

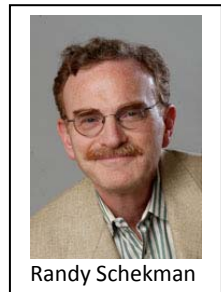


Cells are nature's protein factories. They build enzymes, antibodies, and a host of other critical molecules on a nanoscale assembly line. Berkeley Lab's **Randy Schekman** has devoted his career to researching the intricate systems that pack and transport these proteins between organelles and beyond the cell walls. Schekman, a guest Faculty in Life

Sciences, is an investigator of the Howard Hughes Medical Institute and a Professor of Cell and Developmental Biology in the Department of Molecular and Cell Biology at the University of California at Berkeley.

More> <http://sciencematters.berkeley.edu/archives/volume6/issue43/story1.php>

*Today at Berkeley Lab, CG, 4/17/09*



Randy Schekman

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## Awards

### Kohwi-Shigematsu Wins NIH Roadmap Grant

**Terumi Kohwi-Shigematsu** received a five year NIH Roadmap grant on Epigenomics of Human Health and Disease, awarded by the NIH Roadmap's Epigenomics Program. Mounting evidence suggests that cancer is not necessarily a result of accumulated genetic mutations, but may also involve alterations in the epigenome, the modifications on the DNA or the proteins that package the DNA. In fact, recent

evidence suggests that these modifications are different between metastatic and non-metastatic breast cancer. Kohwi-Shigematsu's lab will determine what epigenomic changes underlie metastatic breast cancer and the mechanisms by which these changes are established.

The overall hypothesis of the NIH Roadmap Epigenomics Program is that the origins of health and susceptibility to disease are, in part, the result of epigenetic regulation of the genetic blueprint. Specifically, epigenetic mechanisms that control stem cell differentiation and organogenesis contribute to the biological response to endogenous and exogenous forms of stimuli that result in disease.

The NIH Roadmap is a series of programs designed to foster new ways of doing research, to fill fundamental knowledge gaps, and to encourage risk taking to solve complex problems. The overarching criterion for Roadmap programs is that they are expected to transform the way research is conducted across the spectrum of health research. The programs in their entirety therefore do not address specific diseases, although individual awards within a program may be disease-specific (<http://nihroadmap.nih.gov/>).

*Terumi Kowhi-Shigematsu, 4/09*

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### Second Place in ABI SOLiD System Genome Grant Program

A research group, spearheaded by **Roger Hoskins** of the Life Sciences Genome Dynamics Department, submitted a proposal, "Comprehensive Characterization of the Drosophila Transcriptome," to Applied Biosciences, Inc. (ABI) for their SOLiD™ System \$10K Genome Grant Program and was awarded second prize. By placing second, Berkeley Lab won free library constructions, sequencing and alignments. ABI will also sequence 12 Drosophila melanogaster embryonic samples for Berkeley Lab at no cost.

The SOLiD™ System is a highly accurate, massively parallel next-generation sequencing platform that supports a wide range of applications. The flexibility of two independent flow cells and multiplexing capability allow a researcher to conduct multiple experiments in a single run. The research group included: **Roger Hoskins** and **Susan Celniker**, Berkeley Lab; Peter Cherbas, Indiana University, Bloomington, IN; Thomas R. Gingeras, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; Michael Brent, Washington University, St. Louis, MO; Brenton R. Graveley, University of Connecticut Health Center, Farmington, CT; Steven E. Brenner, University of California, Berkeley, CA and Norbert Perrimon, Harvard University, Cambridge, MA.

*Susan Celniker, 4/09*

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### Recent publications (selected)

Handford JI, Ize B, Buchanan G, **Butland GP**, Greenblatt J, Emili A, Palmer T. Conserved network of proteins essential for bacterial viability. *Journal of Bacteriology*, 2009 Apr 17. [Epub ahead of print] PMID: 19376873

The yjeE, yeaZ and ygjD genes are highly conserved in the genomes of eubacteria, and ygjD orthologs are also found throughout the Archaea and eukaryotes. In this study we have constructed conditional expression strains for each of these genes in the model organism Escherichia coli K12. We show that each gene is essential for viability of E. coli under laboratory growth conditions. Growth of the conditional

strains under non-permissive conditions results in dramatic changes in cell ultrastructure. Strains depleted for *yeaZ* show highly condensed nucleoids, whilst strains depleted for *yjeE* and *ygjD* show at least a proportion of very enlarged cells with an unusual peripheral distribution of DNA. Each of the three conditional expression strains can be complemented by multicopy clones harboring the *rstA* gene which encodes a two-component system response regulator, strongly suggesting that these proteins are involved in the same essential cellular pathway. Bacterial two hybrid experiments show that YeaZ can interact with both YjeE and YgjD but that YeaZ is the preferred interaction partner. In vitro experiments indicate that YeaZ mediates the proteolysis of YgjD, suggesting that YeaZ and YjeE act as regulators to control the activity of this protein. Our results are consistent with these proteins forming a link between DNA metabolism and cell division.

Weber GH, Rübél O, **Huang MY**, Depace AH, Fowlkes CC, Keränen SV, Luengo Hendriks CL, Hagen H, **Knowles DW**, Malik J, **Biggin MD**, Hamann B. Visual exploration of three-dimensional gene expression using physical views and linked abstract views. *IEEE/ACM Transactions on Computational Biology and Bioinformatics*, 2009 Apr-Jun;6(2):296-309. PMID: 19407353

During animal development, complex patterns of gene expression provide positional information within the embryo. To better understand the underlying gene regulatory networks, the Berkeley Drosophila Transcription Network Project (BDTNP) has developed methods that support quantitative computational analysis of three-dimensional (3D) gene expression in early Drosophila embryos at cellular resolution. We introduce PointCloudXplore (PCX), an interactive visualization tool that supports visual exploration of relationships between different genes' expression using a combination of established visualization techniques. Two aspects of gene expression are of particular interest: 1) gene expression patterns defined by the spatial locations of cells expressing a gene and 2) relationships between the expression levels of multiple genes. PCX provides users with two corresponding classes of data views: 1) Physical Views based on the spatial relationships of cells in the embryo and 2) Abstract Views that discard spatial information and plot expression levels of multiple genes with respect to each other. Cell Selectors highlight data associated with subsets of embryo cells within a View. Using linking, these selected cells can be viewed in multiple representations. We describe PCX as a 3D gene expression visualization tool and provide examples of how it has been used by BDTNP biologists to generate new hypotheses.

Schober D, Smith B, **Lewis SE**, Kusnierczyk W, Lomax J, **Mungall C**, Taylor CF, Rocca-Serra P, Sansone SA. Survey-based naming conventions for use in OBO Foundry ontology development. *BMC Bioinformatics*, 2009 Apr 27;10(1):125. [Epub ahead of print] PMID: 19397794

**BACKGROUND:** A wide variety of ontologies relevant to the biological and medical domains are available through the OBO Foundry portal, and their number is growing rapidly. Integration of these ontologies, while requiring considerable effort, is extremely desirable. However, heterogeneities in format and style pose serious obstacles to such integration. In particular, inconsistencies in naming conventions can impair the readability and navigability of ontology class hierarchies, and hinder their alignment and integration. While other sources of diversity are tremendously complex and challenging, agreeing a set of common naming conventions is an achievable goal, particularly if those conventions are based on lessons drawn from pooled practical experience and surveys of community opinion. **RESULTS:** We summarize a review of existing naming conventions and highlight certain disadvantages with respect to general applicability in the biological domain. We also present the results of a survey carried out to establish which naming conventions are currently employed by OBO Foundry ontologies and to determine what their special requirements regarding the naming of entities might be. Lastly, we propose an initial set of typographic, syntactic and semantic conventions for labelling classes in OBO Foundry ontologies. **CONCLUSIONS:** Adherence to common naming conventions is more than just a matter of aesthetics. Such conventions provide guidance to ontology creators, help developers avoid flaws and inaccuracies when editing, and

especially when interlinking, ontologies. Common naming conventions will also assist consumers of ontologies to more readily understand what meanings were intended by the authors of ontologies used in annotating bodies of data.

**Weiszmann R, Hammonds AS, Celniker SE.** Determination of gene expression patterns using high-throughput RNA in situ hybridization to whole-mount *Drosophila* embryos. *Nature Protocols*, 2009;4(5):605-18. PMID: 19360017

We describe a high-throughput protocol for RNA in situ hybridization (ISH) to *Drosophila* embryos in a 96-well format. cDNA or genomic DNA templates are amplified by PCR and then digoxigenin-labeled ribonucleotides are incorporated into antisense RNA probes by in vitro transcription. The quality of each probe is evaluated before ISH using a RNA probe quantification (dot blot) assay. RNA probes are hybridized to fixed, mixed-staged *Drosophila* embryos in 96-well plates. The resulting stained embryos can be examined and photographed immediately or stored at 4 degrees C for later analysis. Starting with fixed, staged embryos, the protocol takes 6 d from probe template production through hybridization. Preparation of fixed embryos requires a minimum of 2 weeks to collect embryos representing all stages. The method has been used to determine the expression patterns of over 6,000 genes throughout embryogenesis.

**Williams PT.** Incident diverticular disease is inversely related to vigorous physical activity. *Medicine and Science in Sports and Exercise*, 2009 Apr 3. [Epub ahead of print] PMID: 19346983

**PURPOSE:** In 1995, the Health Professionals Follow-up Study published an isolated report of lower diverticular disease risk in physically active men, particularly among those who ran. The purpose of this article was to assess whether this finding can be verified among older men and women of the National Runners' Health Study. **METHODS::** Survival analyses were applied to incident disease occurring during 7.7 yr of follow-up in 9072 men and 1664 women, representing 84% follow-up of the original  $\geq 50$ -yr-old cohort. In addition to the usual running distance (km.d), 80% of the baseline respondents included 10-km footrace performance times (a measure of cardiorespiratory fitness). Results were adjusted for age, sex, and reported intakes of meat, fish, fruit, and alcohol. **RESULTS:** A total of 127 men and 21 women reported clinically diagnosed diverticular disease since baseline. The risk for incident diverticular disease decreased 6.2% per km.d run ( $P = 0.04$ ). Relative to men and women who ran  $\leq 2$  km.d, those who ran an average of  $> 8$  km.d had 48% lower risk ( $P = 0.05$ ). Each meter-per-second increment in the 10-km performance was associated with a 68% risk reduction ( $P = 0.04$ ). Men and women who ran  $> 4$  m.s had 70% lower risk for diverticular disease than those who ran  $\leq 2.8$  m.s ( $P = 0.01$ ), which persisted when adjusted for baseline body mass index (69% risk reduction,  $P = 0.02$ ) or usual running distance (36% risk reduction,  $P = 0.03$ ). **CONCLUSION:** These results demonstrate an inverse association between vigorous physical activity and incident diverticular disease among older men and women but are limited by their reliance on self-reported physician diagnosis.