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Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)

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Disclaimer

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A. OVERALL COVER PAGE

Project Title: Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)	
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Human Subjects: Yes HS Exempt: Yes Exemption Number: E4 Phase III Clinical Trial:	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. OVERALL ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The NIH Research Resource for Biomedical AMS was originally funded at Lawrence Livermore National Laboratory in 1999 to develop and apply the technology of accelerator mass spectrometry (AMS) in broad- based biomedical research. The Resource's niche is to fill needs for ultra high sensitivity quantitation when isotope-labeled agents are used. The Research Resource's Technology Research and Development (TR&D) efforts will focus on the needs of the biomedical research community in the context of seven Driving Biomedical Projects (DBPs) that will drive the Center's technical capabilities through three core TR&Ds. We will expand our present capabilities by developing a fully integrated HPLC AMS to increase our capabilities for metabolic measurements, we will develop methods to understand cellular processes and we will develop and validate methods for the application of AMS in human studies, which is a growing area of demand by collaborators and service users. In addition, we will continue to support new and ongoing collaborative and service projects that require the capabilities of the Resource. The Center will continue to train researchers in the use of the AMS capabilities being developed, and the results of all efforts will be widely disseminated to advance progress in biomedical research. Towards these goals, our specific aims are to:1.) Increase the value and information content of AMS measurements by combining molecular speciation with quantitation of defined macromolecular isolates. Specifically, develop and validate methods for macromolecule labeling, characterization and quantitation.2.) Develop and validate methods and strategies to enable AMS to become more broadly used in human studies. Specifically, demonstrate robust methods for conducting pharmacokinetic/pharmacodynamics studies in humans and model systems.3.) Increase the accessibility of AMS to the Biomedical research community and the throughput of AMS through direct coupling to separatory instruments.4.) Provide high throughput 14C BioAMS analysis for collaborative and service clients.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: overall accomplishments research highlights2016.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

See Dissemination component.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

During the next reporting period, the Research Resource's Technology Research and Development (TR&D) efforts will continue to focus on the needs of the biomedical research community in the context of seven Driving Biomedical Projects (DBPs). We will continue to validate the new BioAMS deck and train our staff and collaborators in its use. Our method development efforts will be focused on HPLC-AMS compatible methods that provide insight into cellular processes and human physiology. In addition, we will continue to recruit and support new and ongoing collaborative and service projects that require the capabilities of the Resource. The Center will continue to train researchers in the use of the AMS capabilities being developed, and the results of all efforts will be widely disseminated to advance progress in biomedical research.
More specific goals for next year are outlined in each of the components.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

The bioAMS Research Resource is focused on the continued growth and application of AMS in biomedical research.

We continue to believe that the primary obstacle to the growth and application of AMS in biomedical research is the lack of high throughput analysis coupled to molecular speciation. This hurdle can be overcome by the development of an on-line fully integrated AMS-HPLC system, which is nearly completed. A second challenge is the lack of robust methods for quantitative protein biochemistry studies. Being able to measure post-translational modifications, perform studies for receptor identification based on binding of ^{14}C-labeled ligands with acceptable sensitivity, precision and reproducibility and use substrate concentrations that are within the natural physiological range is essential. Likewise, we are developing methods to conduct quantitative pharmacokinetic and metabolism studies for biologic drugs, and for fundamental research in protein turnover by our collaborators. We continue to develop separation protocols to utilize reduced sample sizes for bomb pulse macromolecular turnover studies. All these studies are enabled by the new HPLC-AMS system. Third, with the growth in interest in human subjects research and more rapidly developing therapeutics and diagnostics, the ability to measure biological endpoints in humans is important. Robust methods are being developed and validated so that pharmacology, toxicology or basic disease mechanisms investigations can proceed safely in humans.

A major accomplishment toward addressing these challenges was the procurement of a new high voltage AMS deck. This AMS instrument was installed in a biomedical laboratory setting capable of handling Risk Group 2 (RG2) samples. It is being developed to have two liquid sample interfaces that can be easily operated by biomedical scientists. Our overall technological goal in this upcoming cycle is to transition from solid sample AMS analysis to liquid sample AMS analysis and we are making progress towards this goal. This will increase the throughput and ease of use of AMS through direct coupling to separatory instruments and enable us and our collaborators to more readily obtain high-density data sets. This will enable us to develop AMS and apply its quantitative capabilities to enable, effective broad based research in fundamental biology and human health.

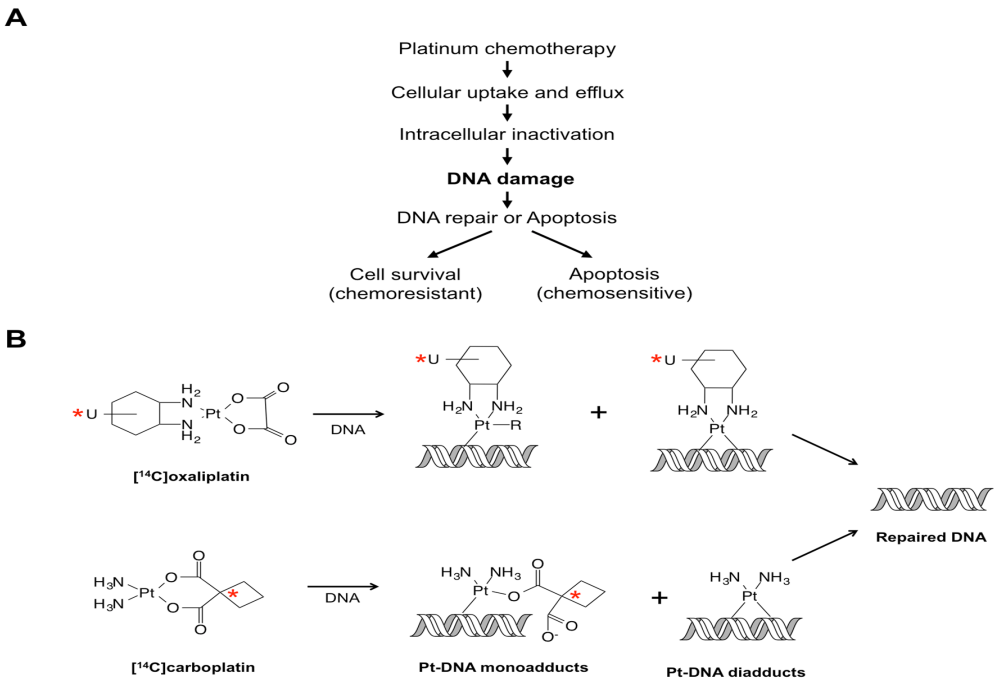
For this funding cycle, the center is organized around 7 driving biomedical projects (DBPs) that are tightly coupled to 3 Core Research and Development (R&D) projects along with a Collaborative and Service Center. Core TR&D Project 1 focuses on increasing the value and information content of AMS measurements by combining molecular speciation with quantitation of defined macromolecular isolates. Our hypothesis is that improved information content in terms of molecule-specific quantitation will result in expanded use of AMS to understand biological mechanisms in health and disease. Specifically, it will develop and validate methods for macromolecule labeling, characterization and quantitation that have become an important need for a number of our collaborators. Core TR&D Project 2 focuses on developing and validating methods to conduct studies in humans, mostly for use in drug development and toxicology. This project is working to test the hypothesis that AMS data can allow earlier than now possible testing of new drugs or fundamental mechanistic questions in humans for understanding human effects of drugs/toxicants or nutrients (specific aims 2 and 3). A number of our collaborators need the ability to judge whether animal models reflect the human situation and AMS offers the capability to specifically address this controversy. Core TR&D Project 3 focuses on our aim to increase AMS throughput via transitioning to liquid sample AMS analysis and through development of a coupled AMS-HPLC system (specific aim 3). Our hypothesis is that such a coupled system will significantly increase throughput and enable routine quantification of individual components of complex mixtures thus broadening AMS's utility for the biomedical sciences. These core projects are linked to develop the technology to allow more rapid and cost effective analysis of metabolism in cells and higher organisms for use in understanding the dynamic processes that occur and how individual variation, disease, toxicants and drugs affect them. The progress of each of these cores and their coupled driving biomedical projects is addressed in the "progress section" of each of their components.

Below we highlight accomplishments in three relevant research areas: 1) induced platinum resistance in a bladder cancer cell model, 2) nonlinear protective effects of resveratrol for cancer chemoprevention and 3) the critical role of histone turnover in neurons for cell-type specific gene expression.

Research Highlights:

Highlight #1 - Induced Platinum Resistance in a Bladder Cancer Cell Model

This study focuses the development, functional and molecular characterization of an isogenic, paired bladder cancer cell culture model system for studying platinum drug resistance. The 5637 human bladder cancer cell line was cultured over ten months with stepwise increases in oxaliplatin concentration to generate a drug resistant 5637R sub cell line. The MTT assay was used to measure the cytotoxicity of several bladder cancer drugs. Liquid scintillation counting allowed quantification of cellular drug uptake and efflux of radiolabeled oxaliplatin and carboplatin. The impact of intracellular drug inactivation was assessed by chemical modulation of glutathione levels. Oxaliplatin- and carboplatin-DNA adduct formation and repair was measured using accelerator mass spectrometry. Resistance factors including apoptosis, growth factor signaling and others were assessed with RNAseq of both cell lines and included confirmation of selected transcripts by RT-PCR. Oxaliplatin, carboplatin, cisplatin and gemcitabine were significantly less cytotoxic to 5637R cells compared to the 5637 cells. In contrast, doxorubicin, methotrexate and vinblastine had no cell line dependent difference in cytotoxicity. Upon exposure to therapeutically relevant doses of oxaliplatin, 5637R cells had lower drug-DNA adduct levels than 5637 cells. This difference was partially accounted for by pre-DNA damage mechanisms such as drug uptake and intracellular inactivation by glutathione, as well as faster oxaliplatin-DNA adduct repair. In contrast both cell lines had no significant differences in carboplatin cell uptake, efflux and drug-DNA adduct formation and repair, suggesting distinct resistance mechanisms for these two closely related drugs. The functional studies were augmented by RNAseq analysis, which demonstrated a significant change in expression of 83 transcripts, including 50 known genes and 22 novel transcripts. Most of the transcripts were not previously associated with bladder cancer chemoresistance. This model system and the associated phenotypic and genotypic data has the potential to identify some novel details of resistance mechanisms of clinical importance to bladder cancer.



DNA damage as the critical step in Pt-induced cell death. (A) The major pathways of platinum (Pt) drug-induced cell death. After administration, cellular uptake and efflux determines the intracellular accumulation of Pt agents, which can be inactivated by the intracellular thiol-containing molecules.

Eventually, Pt agents induce DNA damage, including drug-DNA adducts, which triggers cell cycle arrest and DNA repair. DNA adduct formation and repair determines the fate of cells, although other factors also play important roles, such as pro- and anti-apoptotic proteins. (B) Diagram showing the formation of carboplatin- and oxaliplatin-DNA adducts and the positions of the radiocarbon labels on each drug used for this study in order to enable quantification of drug-DNA adduct formation and repair by accelerator mass spectrometry.

Wang et al., Molecular Dissection of Induced Platinum Resistance through Functional and Gene Expression Analysis in a Cell Culture Model of Bladder Cancer. *PLoS One*. 2016;**11(1)**:e0146256. PMCID: PMC4723083

Highlight #2 – Nonlinear Protective Effects of Resveratrol for Cancer Chemoprevention

Resveratrol is widely promoted as a potential cancer chemopreventive agent, but a lack of information on the optimal dose prohibits rationally designed trials to assess efficacy. To challenge the assumption that "more is better," we compared the pharmacokinetics and activity of a dietary dose with an intake 200 times higher. The dose-response relationship for concentrations generated and the metabolite profile of [^{14}C]-resveratrol in colorectal tissue of cancer patients helped us to define clinically achievable levels. In ApcMin mice (a model of colorectal carcinogenesis) that received a high-fat diet, the low resveratrol dose suppressed intestinal adenoma development more potently than did the higher dose. Efficacy correlated with activation of adenosine monophosphate–activated protein kinase (AMPK) and increased expression of the senescence marker p21. Nonlinear dose responses were observed for AMPK and mechanistic target of rapamycin (mTOR) signaling in mouse adenoma cells, culminating in autophagy and senescence. In human colorectal tissues exposed to low dietary concentrations of resveratrol ex vivo, we measured enhanced AMPK phosphorylation and autophagy. The expression of the cytoprotective NAD(P)H dehydrogenase, quinone 1 (NQO1) enzyme was also increased in tissues from cancer patients participating in our [^{14}C]-resveratrol trial. These findings warrant a revision of developmental strategies for diet-derived agents designed to achieve cancer chemoprevention.

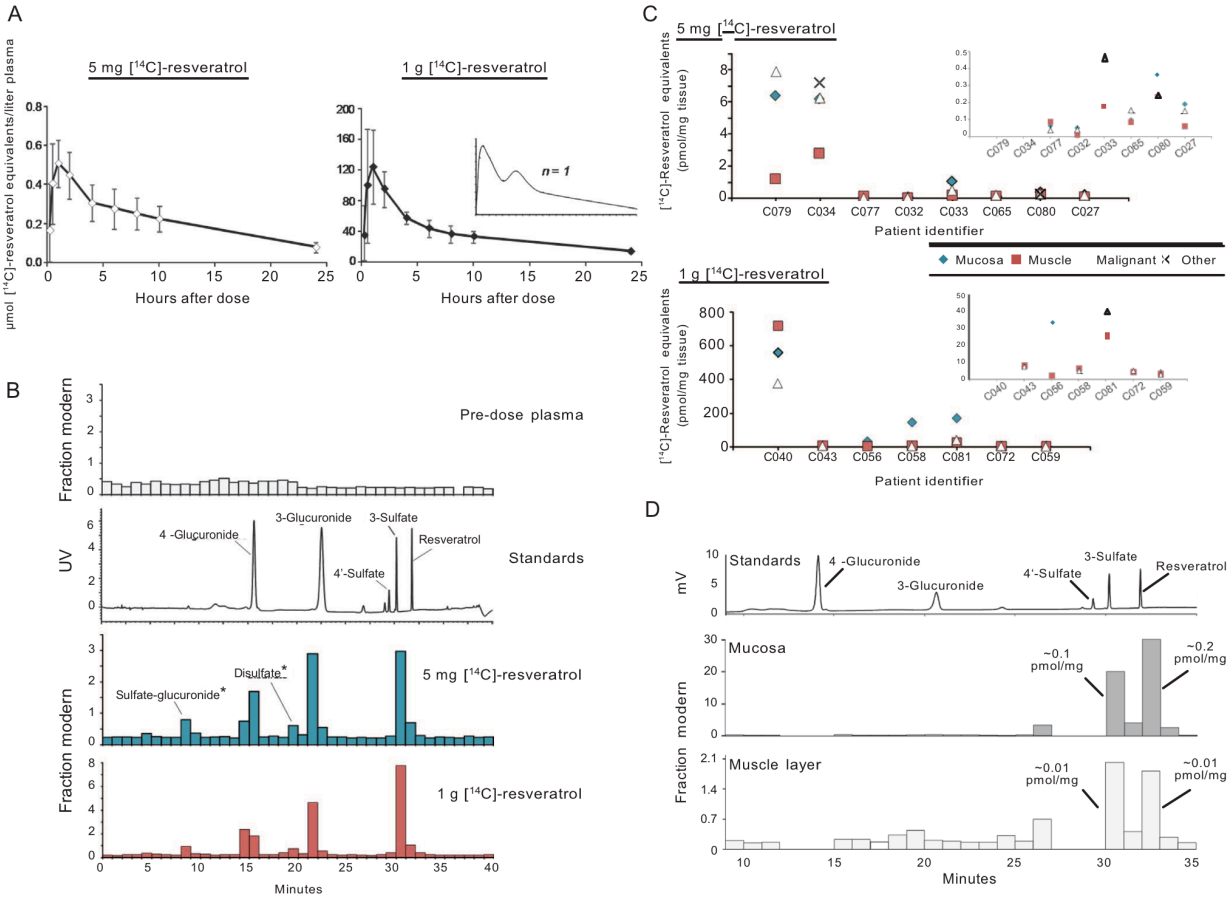


Fig. 1. Quantitation of resveratrol and its metabolites in humans after a low dietarily achievable dose or high pharmacological dose. (A and B) Healthy volunteers received a single [^{14}C]-labeled oral dose of either 5 mg or 1.005 g resveratrol (44.5 kBq, 0.962 mSv), and plasma samples were taken over 24 hours for determination of total [^{14}C]-resveratrol equiv- alents by AMS analysis. (A) Graphs show average (±SD) concentrations for 10 volunteers per group, whereas the inset

represents a single participant to illustrate the second peak maximum commonly observed with resveratrol due to enterohepatic recirculation. (B) Plasma metabolite profiles determined by HPLC-AMS analysis of selected samples from one patient on each resveratrol dose, taken 1 hour after ingestion. Also included are a pre-dose plasma sample for determination of background levels of radio- carbon and an ultraviolet (UV) chromatogram from the analysis of authentic metabolite standards. Peaks designated by * were tentatively assigned on the basis of their chromatographic properties because synthetic standards were not available. (C) Levels of [^{14}C]-resveratrol equivalents in tissues of pa-tients with colorectal cancer that received either 5 mg (n = 8) or 1 g (n = 7) resveratrol daily for 1 week before surgery. Enlargements are included as insets to enable comparisons at lower concen- trations. (D) Metabolite profile in colorectal mucosa and muscle tissue of a patient that received 5 mg of [^{14}C]-resveratrol, determined by HPLC-AMS analysis. Peaks of radiocarbon in both tissue types correspond to resveratrol and its 3-sulfate metabolite, on the basis of the similarity of retention times to authentic standards, and the concentrations stated translate to micromolar, assuming that 1 g of tissue equates to 1 ml.

Cai et al., Cancer chemoprevention: Evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice. *Sci Transl Med*. 2015; **7(298)**: 298ra117

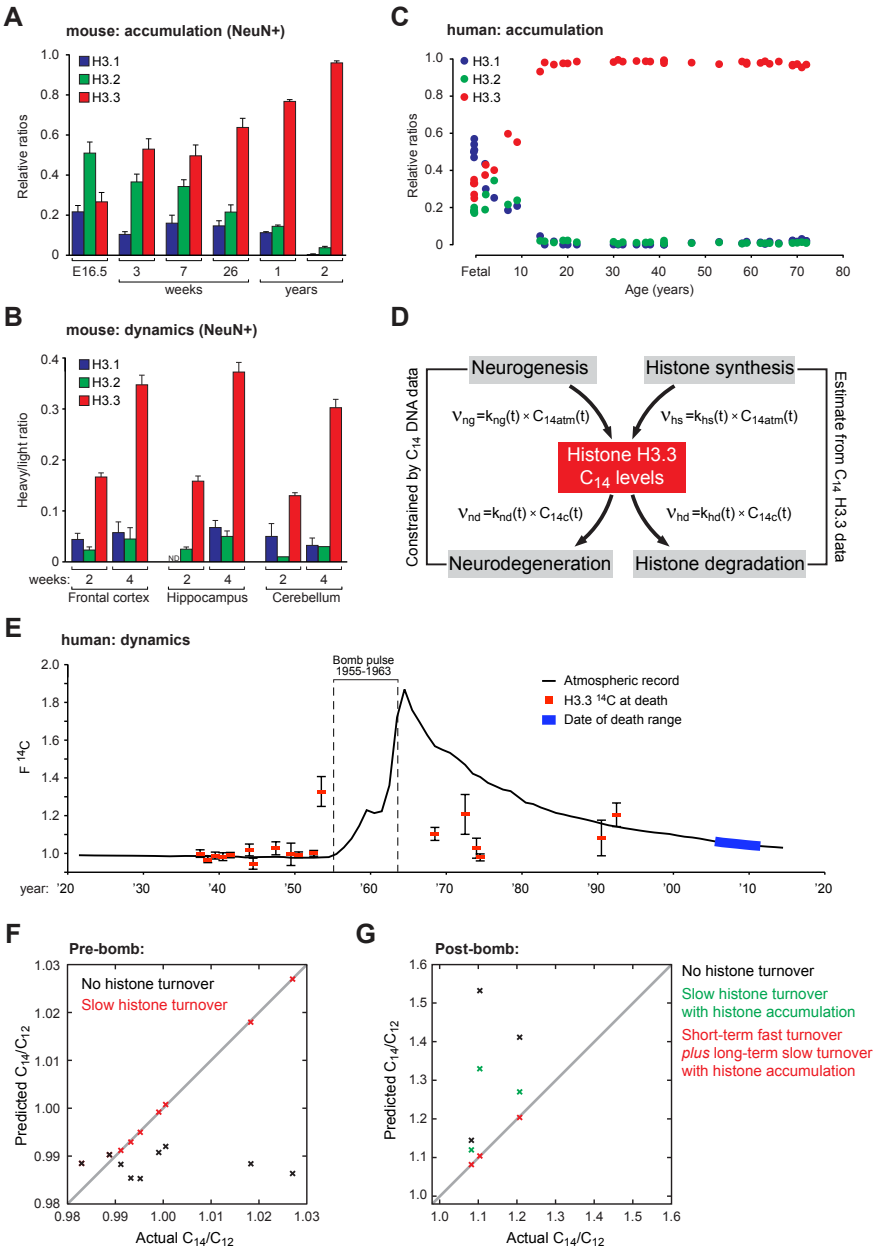
Highlight #3 – Crtitcal Role of Histone Turnover in Neurons

Turnover and exchange of nucleosomal histones and their variants, a process long believed to be static in post-replicative cells, remains largely unexplored in brain. Here, we describe a novel mechanistic role for HIRA (histone cell cycle regulator) and proteasomal degradation-associated histone dynamics in the regulation of activity-dependent transcription, synaptic connectivity, and behavior. We uncover a dramatic developmental profile of nucleosome occupancy across the lifespan of both rodents and humans, with the histone variant H3.3 accumulating to near-saturating levels throughout the neuronal genome by mid-adolescence. Despite such accumulation, H3.3-containing nucleosomes remain highly dynamic—in a modification-independent manner— to control neuronal- and glial-specific gene expression patterns throughout life. Manipulating H3.3 dynamics in both embryonic and adult neurons confirmed its essential role in neuronal plasticity and cognition. Our findings establish histone turnover as a critical and previously undocumented regulator of cell type-specific transcription and plasticity in mammalian brain.

Figure 1. H3.3 accumulates with age but remains dynamic in the CNS
(A) LC-MS/MS quantified H3.1 and H3.2 *vs.* H3.3 protein expression in NeuN+ mouse chromatin with age. (frontal cortex, *n* = 3 biological replicates/age). **(B)** SILAC LC-MS/MS analysis of H3.1/2 *vs.* H3.3 in NeuN+ mouse chromatin from multiple brain structures after two or 4 wks of feeding on a heavy lysine (6 Da) diet. (*n* = 3-6 biological replicates/brain region). **(C)** LC-MS/MS quantified H3.1/2 *vs.* H3.3 protein expression in fetal (*n* = 5) *vs.* adult (*n* = 30) human postmortem brain. **(D)** The rate of change of C14 levels in Histone H3.3 pools from brain tissue depends on the rates of histone H3.3 production and degradation. We model production rates as proportional to the current atmospheric ^{14}C/^{12}C levels, and degradation rates as proportional to current cellular ^{14}C/^{12}C levels. **(E)** F^{14}C (^{14}C/^{12}C) bomb pulse accelerator mass spectrometry of HPLC purified H3.3 protein from postmortem human brain (*n* = 18). Human H3.3 F^{14}C levels (red squares) are plotted against the atmospheric record for ^{14}C (black line) as a function of time (each *n* is plotted along the x-axis by respective dates of birth). The range of death dates is indicated (blue line). 'Error' bars represent one σ deviation above the average for each sample. Modern F^{14}C correction assessments were made using H3.3 protein purified from fetal human brain collected in 2012 (mid-gestation). The atmospheric record of F^{14}C was constructed from published tree ring and CO_2 data of Northern Hemisphere growing season averages (see Supplemental Information for references). **(F and G)** Mathematical descriptions of ^{14}C/^{12}C dynamics in cerebellar H3.3 pools were used to predict ^{14}C/^{12}C ratios, given various assumptions with regards to the nature of H3.3 turnover in healthy post-mitotic

cells, and then compared to observed data. Data represented as means ± SEM, unless otherwise noted.

FIGURE 1



Maze et al., Critical Role of Histone Turnover in Neuronal Transcription and Plasticity. *Neuron* 2015; **87(1)**:77-94. PMCID: PMC4491146

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

Resource staff and associated collaborators engaged in multiple training and professional development activities. These are detailed below, reported for each specific Resource component:

TR&D 1:
Nothing to report

TR&D2:
Hosted two student interns between 06/2015 -08/2015.

TR&D 3:
We provided one-on-one training in the operation of the moving wire interface to Resource staff and collaborators, conducting research in an NIEHS-funded study on the pharmacokinetics of PAHs in human volunteers. We have trained Resource staff on the operation of our new AMS spectrometer and moving wire interface operation. Such training entails the one-on-one mentoring on the procedures needed to successfully load samples into the ion source, turn on and tune the spectrometer, configure the data acquisition system to measure the samples to defined performance criteria.

DBP1:
Nothing to report

DBP2:
Nothing to report

DBP3: Dr. Kin Sing Lee, currently an Asst. Project Scientist in Prof. Bruce Hammock's Lab, is focusing on this project and worked with RR staff member Bruce Buchholz to develop the project into a K99/R00 application. Dr. Lee is in the second year of his K99 and Dr. Buchholz is part of his mentoring team with Prof. Hammock. Dr. Lee is currently interviewing for faculty positions.

DBP4:
Nothing to report

DB5:
Oregon State University SRP trainee Erin Madeen has visited CAMS on several occasions to prep, process, and analyze DBC plasma and urine samples under the mentorship of Resource staff. While analyzing data generated by LS-AMS, Resource Investigator, Dr. Ognibene, developed a user driven pipeline for LS-AMS data that met Erin's needs for quality and uniformity in PK analysis. She shared the dibenzo[def,p]chrysene (DBC) and BaP metabolite data with Rick Corley and Jordan Smith of Pacific Northwest National Laboratory, where she learned basic pharmacokinetic analysis. The skills that she gained during this experience contributed to the successful defense of her Ph.D. dissertation and her recruitment for a post doctoral position in drug metabolism.

Erin Madeen presented a poster at the Gordon Research Conference on Drug Metabolism in Holderness, NH on July 17, 2015 entitled, "Environmentally Relevant Microdosing of Human Volunteers For PAH Pharmacokinetics."

Oregon State University research faculty, Sharon Krueger, became proficient in FDA IND applications and human subjects clinical coordination through her affiliation with the Williams lab. She completed ethics and confidentiality training in addition to bridging relationships and collaboration with the IRB and FDA systems.

OSU undergraduate research assistant and dietetics student, Jessica Hummel, applied her training to analysis of dietary records kept by human subjects to assess compliance with dietary restrictions and to estimate the dietary intake of BaP by subjects enrolled in this project.

A poster was presented at OSU Environmental & Molecular Toxicology Research Day on January 21, 2016 entitled, “Estimated Dietary Exposure to Benzo[a]pyrene (BaP) During Clinical Dietary Restriction,” by Jessica M. Hummel, Erin P. Madeen, Lisbeth K. Siddens, Sandra Uesugi, Sharon K. Krueger, and David E. Williams.

OSU undergraduate research assistants Alex Van Scoyk, Hannah You, David Sampson, and Jessica Hummel, received blood borne pathogens training and training in sample preparation through their involvement in SRP Project 1.

DBP 6:

UC Davis Postdoctoral Fellow Maike Zimmermann was awarded a travel grant to attend and present at the 2015 “Association for Mass Spectrometry: Applications to the Clinical Lab” meeting, which will be held in San Diego on March 28, 2015.

UC Davis Postdoctoral Fellow Tiffany Scharadin was trained in clinical leukemia research methods in order to pursue feasibility experiments in a leukemia cell culture model system.

UC Davis Medical School Professor Brian Jonas participated in an internal grant proposal with Mike Malfatti (TR&D2) in autumn of 2015. Although the grant was not funded, it formed a roadmap for studying chemoresistance in leukemia that is now under active investigation on clinical samples. **Notably, Dr. Jonas is an NIH K12 funded investigator, and is using his funding to pursue AMS studies of leukemia.**

DBP7:
Three members of our group [two graduate students and a technician] participated in the UC Davis Cancer Center Symposium where they presented two posters and had the opportunity to interact with clinical oncologists and discuss collaborative studies and extensions of the developed application of C-14 labeling in xenografts.

CP1:
Nothing to report

CP2:
Nothing to report

CP3:

Development of the elastin purification protocol was an iterative process between forensic medicine doctor (Dr. Christian Matzenauer) in Düsseldorf, Germany and Bruce Buchholz at LLNL.

CP4:
Heather Enright attended two conferences last year to present this work.

CP5:
Nothing to Report

CP6:
Alan McCartt began training for the purification of gaseous biological samples.

CP7:
Nothing to Report

Other collaborations:

Dr. Aboud completed a postdoctoral fellowship involving work at LLNL performing metabolic tracer experiments and sample preparation procedures for AMS analyses.

C. OVERALL PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Boström E, Westerlund I, Vial C, Buchholz BA, Possnert G, Mash DC, Druid H, Frisén J. Dynamics of hippocampal neurogenesis in adult humans. Cell. 2013 Jun 6;153(6):1219-27. PubMed PMID: 23746839; PubMed Central PMCID: PMC4394608.
Complete	Etminan N, Buchholz BA, Dreier R, Bruckner P, Torner JC, Steiger HJ, Hänggi D, Macdonald RL. Cerebral aneurysms: formation, progression, and developmental chronology. Transl Stroke Res. 2014 Apr;5(2):167-73. PubMed PMID: 24323717; PubMed Central PMCID: PMC4399795.
Complete	Hudson BD, Hum NR, Thomas CB, Kohlgruber A, Sebastian A, Collette NM, Coleman MA, Christiansen BA, Loots GG. SOST Inhibits Prostate Cancer Invasion. PLoS One. 2015;10(11):e0142058. PubMed PMID: 26545120; PubMed Central PMCID: PMC4636315.
Non-Compliant	Nallathamby PD, Mortensen NP, Palko HA, Malfatti M, Smith C, Sonnett J, Doktycz MJ, Gu B, Roeder RK, Wang W, Retterer ST. New surface radiolabeling schemes of super paramagnetic iron oxide nanoparticles (SPIONs) for biodistribution studies. Nanoscale. 2015 Apr 21;7(15):6545-55. PubMed PMID: 25790032.
Complete	Wettersten HI, Hakimi AA, Morin D, Bianchi C, Johnstone ME, Donohoe DR, Trott JF, Aboud OA, Stirdivant S, Neri B, Wolfert R, Stewart B, Perego R, Hsieh JJ, Weiss RH. Grade-Dependent Metabolic Reprogramming in Kidney Cancer Revealed by Combined Proteomics and Metabolomics Analysis. Cancer Res. 2015 Jun 15;75(12):2541-52. PubMed PMID: 25952651; PubMed Central PMCID: PMC4470795.
Complete	Maze I, Wenderski W, Noh KM, Bagot RC, Tzavaras N, Purushothaman I, Elsässer SJ, Guo Y, Ionete C, Hurd YL, Tamminga CA, Halene T, Farrelly L, Soshnev AA, Wen D, Rafii S, Birtwistle MR, Akbarian S, Buchholz BA, Blitzer RD, Nestler EJ, Yuan ZF, Garcia BA, Shen L, Molina H, Allis CD. Critical Role of Histone Turnover in Neuronal Transcription and Plasticity. Neuron. 2015 Jul 1;87(1):77-94. PubMed PMID: 26139371; PubMed Central PMCID: PMC4491146.
Non-Compliant	Cai H, Scott E, Kholghi A, Andreadi C, Rufini A, Karmokar A, Britton RG, Horner-Glister E, Greaves P, Jawad D, James M, Howells L, Ognibene T, Malfatti M, Goldring C, Kitteringham N, Walsh J, Viskaduraki M, West K, Miller A, Hemingway D, Steward WP, Gescher AJ, Brown K. Cancer chemoprevention: Evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice. Sci Transl Med. 2015 Jul 29;7(298):298ra117. PubMed PMID: 26223300; NIHMSID: 771973.
Complete	Hooper-Bui LM, Kwok ES, Buchholz BA, Rust MK, Eastmond DA, Vogel JS. Insecticide Transfer Efficiency and Lethal Load in Argentine Ants. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:665-669. PubMed PMID: 26504258; PubMed Central PMCID: PMC4615608.
Complete	Ognibene TJ, Haack KW, Bench G, Brown TA, Turteltaub KW. Operation of the "Small" BioAMS Spectrometers at CAMS: Past and Future Prospects. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:54-57. PubMed PMID: 26456990; PubMed Central PMCID: PMC4594839.

Complete	Ognibene TJ, Thomas AT, Daley PF, Bench G, Turteltaub KW. An Interface for the Direct Coupling of Small Liquid Samples to AMS. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:173-177. PubMed PMID: 26456991; PubMed Central PMCID: PMC4594830.
Non-Compliant	Jiang S, Pan AW, Lin TY, Zhang H, Malfatti M, Turteltaub K, Henderson PT, Pan CX. Paclitaxel Enhances Carboplatin-DNA Adduct Formation and Cytotoxicity. Chem Res Toxicol. 2015 Dec 21;28(12):2250-2. PubMed PMID: 26544157.
In Process at NIHMS	Tong T, Ondov JM, Buchholz BA, VanDerveer MC. Contemporary carbon content of bis (2-ethylhexyl) phthalate in butter. Food Chem. 2016 Jan 1;190:1064-8. PubMed PMID: 26213077; NIHMSID: 771207.
Complete	Wang S, Zhang H, Scharadin TM, Zimmermann M, Hu B, Pan AW, Vinall R, Lin TY, Cimino G, Chain P, Vuyisich M, Gleasner C, Mcmurry K, Malfatti M, Turteltaub K, de Vere White R, Pan CX, Henderson PT. Molecular Dissection of Induced Platinum Resistance through Functional and Gene Expression Analysis in a Cell Culture Model of Bladder Cancer. PLoS One. 2016;11(1):e0146256. PubMed PMID: 26799320; PubMed Central PMCID: PMC4723083.
Non-Compliant	Malfatti MA, Kuhn EA, Turteltaub KW, Vickers SM, Jensen EH, Strayer L, Anderson KE. Disposition of the Dietary Mutagen 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline in Healthy and Pancreatic Cancer Compromised Humans. Chem Res Toxicol. 2016 Mar 21;29(3):352-8. PubMed PMID: 26918625.
In Process at NIHMS	Measurements of Carbon-14 With Cavity Ring-Down Spectroscopy. Nuclear instruments & methods in physics research. Section B, Beam interactions with materials and atoms. NIHMSID: 705983.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

bioams.llnl.gov

The Biomedical AMS Resource maintains a website at http://bioams.llnl.gov/. The website was recently refreshed and updated. It now has a section focusing on the liquid sample interface and a newly updated training section has been added.

The website is a good source of information about the resource, and includes sections that describe recent advances in AMS, recent publications and “AMS in the News”. More importantly, the website acts as an interface between potential AMS users/ collaborators and Resource Staff. The website has information about the Resource, which includes: a description of the Resource analytical equipment, what is BioAMS and how it can be used, a description of liquid sample AMS that includes a video of the interface in operation, the physics of AMS operation, how to visit the resource, and resource staff contact information.

The operational procedures for access to the Resource are available on the website. Prior to formally applying for access to the Resource, an investigator must discuss a proposal concept with an appropriate Resource staff member for evaluation of project compatibility with AMS. Only after pre-screening is an application for access to the Resource submitted to the Resource Administrator. This process has served to eliminate the need to reject applications.

Once a project has been designed the collaborator or service user applies for formal access. In order to obtain access to Resource services, all biomedical researchers will be requested to submit an application, which includes a formal proposal or a letter of intent regarding their scientific problem. A specific explanation of the study requirements is required on the “Application for Access to the NIH Resource for Biomedical AMS” form. Proposals and letters of intent will be able to be submitted at any time. These forms are available on the website.
The Resource is also featured on the Physical and Life Sciences Directorate website at https://www-pls.llnl.gov/?url=about_pls-centers_and_institutes-bioams.

C.3 TECHNOLOGIES OR TECHNIQUES

Research activities funded through this Resource include the development of our Liquid Sample AMS (LS-AMS) measurement capabilities. LS-AMS enables the measurement of biochemicals containing a few 10s of nanograms carbon and as little as 50 zmol of 14C at a fraction of the cost and with a significant reduction in sample preparation time as compared to our traditional method of solid

sample analysis. We share this technology through press releases, presentations at conferences and through visitors to the Resource. In addition, we sponser outside collaborators to come to LLNL and perform research using our technology.

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

File uploaded: Other products C5_A.pdf

C.5.b Resource sharing

File uploaded: Resource And Data Sharing Plan.pdf

C.5 Other Products and Resource Sharing

We have developed a computer program for the analysis of liquid sample AMS data (*LS-AMS Analysis*). The standalone executable program runs on either OSX or Windows PC and is available for collaborators using our moving wire interface for the analysis of small liquid samples. The program is available to users of this Research Resource by contacting Ted Ognibene (resource staff member).

Any genetically modified cell lines associated with DBP7 we generate in this program will be freely available to academic researchers.

Resource And Data Sharing Plan

Appended below is the Data Sharing Plan for the Research Resource. This plan is fully implemented. All data generated for collaborators is shared with that collaborator after analysis. We encourage collaborators to publish the data in a timely fashion and help with manuscript writing and editing where appropriate. Third party requests for large data sets would be handled as described below.

The purpose of this Center is to develop technology and make it accessible to the scientific community for use in investigations that require the capabilities of AMS developed at LLNL. All investigators working as part of this Center are committed to the timely release and sharing of final research data, as well as the protection of the rights and privacy of all research subjects. Data generated as part of our Technology Research and Development efforts will be made available as described below. All requests for data developed as part of a collaboration will be referred to the appropriate PI for release.

All research conducted as part of this center is published in the open literature as soon as possible following completion of the studies. Any request for data sharing is reviewed by the PI, internal executive committee and may be reviewed by the external advisory committee, the institutional IRB and/or the institutions legal department, depending on need. Requestors and the authorized representative of their academic or other institution will be required to sign a simple letter agreement, which describes the proposed research and promises to only use the data for the specific NIH-funded projects described. In addition, requesters will be billed for all out-of-pocket expenses incurred in the preparation of the documentation and data for sharing, including the cost of recording medium and postage.

Data requested will be provided in a computerized form on a CD-ROM or other appropriate storage medium. Any data that will not fit on a CD-ROM or similar storage medium will be copied to a portable hard drive, which will be provided by the requestor of the data. All information needed to access the data will be provided via email or on a CD-ROM, including the type of statistical software used to record and analyze the data and all documentation created including codebooks, naming conventions, explanations of how coding decisions were made and how variables were constructed, which field procedures were used, and how the data was cleaned and formatted.

In the case of human subjects data or proprietary data, requestors will be informed of any redacting of data necessary to protect subjects' identities, personal information and intellectual property. Depending on the research project, the necessary redacting may consist one or more of the following:

• Withhold some part of the data

• Statistical alteration of data in ways that do not compromise secondary analyses but will protect individual subjects' identities

• Restriction of access to the data at a controlled site

• Release of a redacted dataset for general use, and the granting of access of more sensitive data at a controlled site.

Data will be available for sharing for three years following the final closeout of the grant or any retention period required by NIH, whichever is longer.

The efforts of this Center to share information will comply fully with the National Institutes of Health policy on data sharing (NOT-OD-03-032), and will facilitate timely sharing of data, free of identifiers, to colleagues and the public.

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Component(s)	Country	SS
	N	Turteltaub, Kenneth W.		01/1957	BS,MS,PHD	PD/PI	0	0	0				NA
KENNETH_TURTELTAUB	Y	Turteltaub, Kenneth W.		01/1957	BS,MS,PHD	PD/PI	4	0	0				NA
	Y	Bench, Graham			PhD	Co-Investigator	3	0	0		Admin Core-8634 (Administration), Core-8623 (TR&D3 Liquid Sample AMS), Training-8632 (Training)		NA
	Y	Enright, Heather			PhD	Co-Investigator	4	0	0		Core-8622 (TR&D2 Quantitative Endpoints in Humans), Core-8631 (Collaboration and Service)		NA
	N	Haack, Kurt			BS	Technician	12	0	0		Core-8623 (TR&D3 Liquid Sample AMS), Core-8631 (Collaboration and Service), Training-8632 (Training)		NA
	N	Hum, Nicholas			MS	Technician	2	0	0		Project-8630 (DBP7 Development of Quanti…rimary Tumors)		NA
	N	Kuhn, Edward			BS	Technician	8	0	0		Core-8623 (TR&D3 Liquid Sample AMS), Core-8631 (Collaboratio		NA

											n and Service), Project-8629 (DBP6 Microdose-based Predi…rapy Efficacy)		
	Y	Ognibene, Ted			PhD	Co-Investigator	9	0	0		Core-8623 (TR&D3 Liquid Sample AMS), Core-8631 (Collaboratio n and Service), Project-8628 (DBP5 The Pharmacokin etics …mental Levels), Training-8632 (Training)		NA
BJSTEWART66	Y	Stewart, Benjamin J			PhD	Co-Investigator	3	0	0		Core-8621 (TR&D1 Quantitative Protein…ular Turnover), Core-8631 (Collaboratio n and Service), Project-8625 (DBP2 Absolute Quantitation …Modificatio ns), Project-8627 (DBP4 Developmen t of Synthe…utic Proteins)		NA
BRUCE_BUCHHOLZ	Y	Buchholz, Bruce A		01/1961	AB,MS,PHD	Co-Investigator	7	0	0		Admin Core-8634 (Administrati on), Core-8621 (TR&D1 Quantitative Protein…ular Turnover), Core-8631 (Collaboratio		NA

											n and Service), Core-8633 (Disseminati on), Project-8624 (DBP1 Bomb Pulse Biology), Project-8626 (DBP3 Identifying Receptors… drolase (sEH))		
BSIDDO	N	Siddoway, Benjamin			PHD,BS	Postdoctor al Scholar, Fellow, or Other Postdoctor al Position	0	4	0		Core-8631 (Collaboratio n and Service)		NA
DANIEL_M CCARTT	N	McCartt, Alan Daniel			PhD	Postdoctor al Scholar, Fellow, or Other Postdoctor al Position	9	0	0		Core-8631 (Collaboratio n and Service)		NA
EPMADEE N	N	Madeen, Erin	1570	10/1983	BS	Postdoctor al Scholar, Fellow, or Other Postdoctor al Position	0	5	0		Project-8628 (DBP5 The Pharmacokin etics …mental Levels)		NA
GABRIELA _LOOTS	Y	LOOTS, GABRIELA G	2800	05/1974	PHD,BS	Co-Investigator	4	0	0		Core-8631 (Collaboratio n and Service), Project-8630 (DBP7 Developmen t of Quanti…rim ary Tumors)		NA
KRISTEN_ KULP	Y	KULP, KRISTEN S		07/1961	PHD	Co-Investigator	2	0	0		Admin Core-8634 (Administrati on), Core-8633 (Disseminati on), Training-8632 (Training)		NA
MICHAEL_ MALFATTI	Y	Malfatti, Michael A.		06/1965	PHD,MA ,BS	Co-Investigator	4	0	0		Core-8622 (TR&D2 Quantitative Endpoints in Humans),		NA

											Core-8631 (Collaboration and Service), Project-8627 (DBP4 Development of Synthe…utic Proteins), Project-8629 (DBP6 Microdose-based Predi…rapy Efficacy)		
PAUL_HENDERSON	Y	Henderson, Paul Thomas	2375		BS,PHD	Co-Investigator	4	0	0		Core-8622 (TR&D2 Quantitative Endpoints in Humans), Core-8631 (Collaboration and Service), Project-8629 (DBP6 Microdose-based Predi…rapy Efficacy)		NA
SARAHCA	N	Carratt, Sarah Ann			BS,PHD	Graduate Student (research assistant)	8	0	0		Core-8631 (Collaboration and Service)		NA
SKSLEE	N	Lee, Kin Sing Stephen	9551	06/1980	PHD,BS	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	3	0	0		Project-8626 (DBP3 Identifying Receptors… drolase (sEH))		NA
TSCHARADIN	N	Scharadin, Tiffany Marie	1455	06/1985	PHD,BS	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	0	3	0		Project-8629 (DBP6 Microdose-based Predi…rapy Efficacy)		NA
	N	Aboud, Omran			DVM, PhD	Collaborator	0	4	0		Core-8631 (Collaboration and Service)		NA
	N	Bales, Karen			PhD	Collaborator	0	1	0		Core-8631 (Collaboration and Service)		NA
	N	Berger, Patricia			PhD	Collaborator	0	1	0		Core-8631 (Collaboration and		NA

											Service)		
	N	Brown, Karen			PhD	Collaborator	0	2	0	University of Leicester	Core-8631 (Collaboration and Service)	UNITED KINGDOM	NA
	N	Burlingame, Alma			PhD	Collaborator	0	1	0		Project-8625 (DBP2 Absolute Quantitation …Modifications)		NA
	N	Chun, Jerold			PhD	collaborator	0	1	0		Core-8631 (Collaboration and Service)		NA
	N	Cooke, Daniel			MD	Collaborator	0	1	0		Core-8631 (Collaboration and Service)		NA
	N	Dreier, Rita			PhD	Collaborator	0	1	0	Westfalian-Wilhelms University	Core-8631 (Collaboration and Service)	GERMANY	NA
	N	Etminan, Nima			MD	Collaborator	0	2	0	Heidelberg University	Core-8631 (Collaboration and Service)	GERMANY	NA
	N	Hammock, Bruce			PhD	Collaborator	0	1	0		Project-8626 (DBP3 Identifying Receptors… drolase (sEH))		NA
	N	Holden, Patricia			PhD	Collaborator	0	1	0		Core-8631 (Collaboration and Service)		NA
	N	Lam, Kit			MD, PhD	Collaborator	0	1	0		Core-8631 (Collaboration and Service)		NA
	N	Madden, Richard			PhD,BS	Collaborator	0	3	0		Core-8631 (Collaboration and Service)		NA
	N	Pan, Chong-xian			MD, PhD	Collaborator	0	2	0		Core-8631 (Collaboration and Service)		NA
	N	Patel, Ketan			PhD	postdoc at foreign university-no commons id	0	1	0		Core-8631 (Collaboration and Service)		NA
	N	Sarachine-			PhD	Collaborato	0	1	0		Core-8631		NA

		Falso, Miranda				r					(Collaboration and Service)		
	N	Spalding, Kirsty			PhD	Collaborator	0	1	0	Karolinska Institute	Project-8624 (DBP1 Bomb Pulse Biology)	SWEDEN	NA
	N	Van Winkle, Laura			BS,PHD	Collaborator	0	2	0		Core-8631 (Collaboration and Service)		NA
	N	Vander Griend, Donald			PhD	Collaborator	0	1	0		Core-8631 (Collaboration and Service)		NA
	N	Weiss, Robert			MD	Collaborator	0	1	0		Core-8631 (Collaboration and Service)		NA
	N	Williams, David			PhD	Collaborator	0	2	0		Project-8628 (DBP5 The Pharmacokinetics …mental Levels)		NA
	N	Zimmermann, Maike			PhD	Collaborator	0	3	0		Project-8629 (DBP6 Microdose-based Predi…rapy Efficacy)		NA

Glossary of acronyms:

S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: OtherSupport_bioAMS_March2016.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

Principal Investigator: Turteltaub, Kenneth W.

For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel

PHS 398/2590 OTHER SUPPORT

The information in this document represents current Other Support for all Senior/Key Personnel. Changes are highlighted in yellow.

Turteltaub, Kenneth

Active

P41GMI03483 (Turteltaub) 6/1/14- 5/31/19 2.4 Cal Months

NIH/NIGMS $1,466,000

Resource for Development of Biomedical Accelerator Mass Spectrometry
The major goals of this project are to expand the present capabilities of BioAMS by developing a fully integrated HPLC/AMS interface, to study biochemical pathways down to the single cell level, and to validate methods for AMS in human translational research.
Role: Principal Investigator

R21GM111242 (Turteltaub) 9/22/2014 – 6/30/2017 2.4 Cal months
NIH/NIGMS

Development of Laser Spectroscopic Methods For Quantification of ^{14}C
The goal of this project is to develop a spectroscopic instrument to quantify ^{14}C in biological samples.
Role: Principal Investigator

Program Management (Turteltaub) Ongoing 4.8 Cal months
LLNL
Physical & Life Sciences Directorate
LLNL Institutional Support for oversight of Biosciences

Program Management (Turteltaub) Ongoing 2.4 Cal months
LLNL
Global Security Directorate
LLNL Institutional Support for oversight of medical countermeasures

OVERLAP
None

Bench, Graham

Active

P41GMI03483 (Turteltaub) 6/1/14- 5/31/19 2.4 Cal Months

NIH/NIGMS $1,466,000

Resource for Development of Biomedical Accelerator Mass Spectrometry
The major goals of this project are to expand the present capabilities of BioAMS by developing a fully integrated HPLC/AMS interface, to study biochemical pathways down to the single cell level, and to validate methods for AMS in human translational research.
Role: Co-Project Lead

Principal Investigator: Turteltaub, Kenneth W.

LLNL Internal Funds (Bench) 0/1/2013– ongoing 9.6 Cal Months
LLNL/DOE $648,200
CAMS Director and Strategic Mission support
This funding enables Dr. Bench to direct, lead and represent the Center for Accelerator Mass Spectrometry to LLNL and the external community.

OVERLAP
There is no scientific overlap between the two projects

Buchholz, Bruce

ACTIVE

*3 R01 ES020867-02S1 (PIs: Ding, Van Winkle) 5/1/15-4/30/18 1.9 cal. mo.
NIH/NIEHS $250,000
Metabolic Mechanisms of Naphthalene Toxicity in Lung
The goal of Dr. Buchholz's role in AIM 1 is to test the hypothesis that napthalene metabolites will form covalent adducts with DNA. If stable naphthalene-DNA adducts are demonstrated it would drive the risk estimation because this mechanism of action is not considered to have a threshold and is tightly linked to carcinogenesis.
Role: Co-investigator

*1R01EY024607-01 Bassnett (PI) 10/1/14-9/30/17 0.5 cal. mo.
NIH/NEI $11,400 total LLNL/year
Role of Microfibrils in the Biology of Pathology of the Eye
The overall goals of the project are to establish the role of microfibrils in healthy and diseased states of the eye using an animal model. Dr. Buchholz will be determining the age/turnover of human zonules obtained from donated eyes.
Role: Co-investigator

P41GM103483 (PI: Turteltaub) 6/1/14-5/31/19 1.8 cal. mo.
NIH/NIGMS $1,466,000
Resource for the Development of Biomedical Mass Spectrometry
The goal of this proposal is to expand the use of accelerator mass spectrometry in bioscience for NIH projects.
Role: Co-Investigator

CAMS Institutional Operation Bench (Director) Ongoing 7.8 cal. mo.
Lawrence Livermore National Laboratory
Operation, Improvement and Promotion of AMS in Geoscience, Energy, and Bioscience.
The CAMS Institute operates a multipurpose AMS facility and collaborates with several institutions.
Role: Scientific Staff

*Newly funded project

RECENTLY COMPLETED

LC130820 Buchholz (PI) 9/1/14-3/31/16 closed
DoD/CDMRP/LCRP $100,000
Mechanism for Clastrogenic Activity of Naphthalene
The goals of this project are 1) to determine which chemically synthesized NA metabolites can form DNA adducts in vitro with which bases in DNA and 2) Quantify NA-DNA adduct formation in nasal and airway tissue of rats, mice and rhesus monkeys exposed a single time in vitro. Adduct stability is not tested in this project.

Principal Investigator: Turteltaub, Kenneth W.
Role: PI

OVERLAP
none

Enright, Heather

ACTIVE

P41GMI03483 (Turteltaub)	6/1/14- 5/31/19	2.4 Cal Months
NIH/NIGMS	$1,466,000	

Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)

The major goals of this project are to expand the present capabilities of BioAMS by developing a fully integrated HPLC/AMS interface, to study biochemical pathways down to the singe cell level, and to validate methods for AMS in human translational research.

Role:

LDRD 13-ERD-045 (E. Wheeler)	10/1/2013 – 9/30/2016	6.6 Cal Months
LLNL Internal Investment	$2,100,000	

Bioengineering systems for medical countermeasures

The major goal of this project is to develop an in-vitro microfluidics platform that will support the long-term growth of human neurons. The platform will be used to understand mechanisms of toxicant exposure and develop countermeasures.

Role:

DTRA1002714357	Valdez (PI)	10/03/2014-09/30/2017	3.0 Cal Months
DoD		$2,500,000	

Development and Optimization of a Blood-Brain Barrier Permeable AChE Reactivator
The major goal of this project is to develop a novel therapeutic reactivator for nerve agent adducted AChE.
Role:

Henderson, Paul

No change

Kulp, Kristen

ACTIVE

P41GMI03483 (Turteltaub)	6/1/14- 5/31/19	1.2 Cal Months
NIH/NIGMS	$1,466,000	

Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)

The major goals of this project are to expand the present capabilities of BioAMS by developing a fully integrated HPLC/AMS interface, to study biochemical pathways down to the singe cell level, and to validate methods for AMS in human translational research.

LDRD 13-ERD-045 (E. Wheeler)	10/1/2013 – 9/30/2016	3.0 Cal Months
LLNL Internal Investment	$2,100,000	

Bioengineering systems for medical countermeasures

The major goal of this project is to develop an in-vitro microfluidics platform that will support the long-term growth of human neurons. The platform will be used to understand mechanisms of toxicant exposure and develop countermeasures.

LLNL other funding 7.8 Cal Months

Principal Investigator: Turteltaub, Kenneth W.

Funding to perform managerial duties in support of lab programs

OVERLAP
None

Loots, Gabriela

ACTIVE

1 R01 AR064255 (PI: Genetos D)	10/1/2013 – 9/30/2018	0.6 Cal mo.
NIH/NIA-NIAMS	Annual Direct Costs	$250,000

Integration of TGFb-ALK5 on Wnt signaling and mechanotransduction in bone
The goal of this proposal is to determine the role of Wnt and TGFb signaling in mechanotransduction.
OVERLAP: none

8 P41MI03483 (PI: Turteltaub K.)	6/1/2014 – 5/31/2019	1.8 Cal mo.
NIH/NIGMS	Annual Direct Costs	$1,466,000

Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)
The goal of this proposal is to develop AMS methodology and process biomedical samples by AMS to promote biomedical research.
OVERLAP: none

OR130220 (PI: Loots G.)	8/1/2014 – 7/31/2017	2.0 Cal mo.
DOD	Annual Direct Costs	$170,000

Evaluating Efficacy of Novel Therapeutics for Mitigating Post-traumatic Osteoarthritis
Determine Molecular Differences between OA-susceptible (STR/ort) and OA-resistant (MRL/MpJ) mouse strains, in a traumatic OA model; validate the therapeutic potential of Adamts16, Crabp2, Has1, Tmp1 and Wnt16 in traumatic OA.
OVERLAP: none

*LDRD16-ERD-007 (PI: Loots)	1/1/2016 – 9/30/2018	7.6 Cal mo.
LLNL		

Characterizing Host-Pathogen Immunity-Gut-Brain Interactions
The goal of this project is to evaluate the relationship between trauma, the immune system, host gut biome and the central nervous system by combining a well-established mouse fracture model and sepsis model.
Role: PI
OVERLAP: none
*Newly funded project

RECENTLY COMPLETED

5 R01 DK075730 (PI: Loots GG)	7/17/2009 – 5/31/2015	closed
NIH/NIDDK	Annual Direct Costs	$160,000

MECHANISMS OF INHIBITING BONE FORMATION THROUGH ANTAGONISM
The major goal of this project is to understand the intersection between the PTH, WNT and BMP pathways during bone formation and homeostasis and determine what roles of Mef2 transcription regulatory proteins play in modulating Sclerostin expression via a key regulatory element that has been shown to be PTH responsive
OVERLAP: none

13-ERD-042 (PI: Loots GG)	10/1/2012 – 9/30/2015	closed
LLNL/LDRD (idea concept award)	Annual Direct Costs	$300,000

Optimizing Drug Efficacy through Pharmacogenomics-Driven Personalized Therapy

Principal Investigator: Turteltaub, Kenneth W.

The major goal of this project is to determine genetic links to drug and bone metabolism through systematic characterization of PTH drug response in mutant mouse strains.

OVERLAP: none

Malfatti, Michael

ACTIVE

P41GM103483-16 (Turteltaub) 6/1/14- 5/31/19 1.2 Cal months
NIH/NIGMS $1,466,000
Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)
Role: Co-investigator
The major goals of this project are to expand the present capabilities of BioAMS by developing a fully integrated HPLC/AMS interface, to study biochemical pathways down to the single cell level, and to validate methods for AMS in human translational research.

US Department of Defense/LLNL Valdez (PI) 10/01/14-9/31/17 6.0 Cal months
Projects supporting the strategic goals of US DOD.
The goal of this project is to determine the biodistribution and pharmacokinetics of a novel oxime acetylcholinesterase reactivator in a relevant animal model, using accelerator mass spectrometry.
Role: Co-Investigator

*PLS-LDRD: 16-ERD-O07 Loots (PI) 1/1/16-9/30/18 2.4 Cal months
LLNL/DOE
Characterizing Host Pathogen Immunity-Gut-Brain Interactions
The goal of this project is to generate and analyze OMIC data that is derived from in vivo infection models that monitor transcriptional expression in real time in both host and pathogen as a function of disease progression and traumatic injury. The long term goal is to understand mechanisms of tolerance to pathogens, and to identify novel molecules that can be used therapeutically to reduce sepsis in trauma patients.
Role: Co-Investigator

LLNL other funding 2.4 Cal months
Funding to perform duties in support of lab programs

*Newly funded project

OVERLAP
None

RECENTLY COMPLETED
PLS-LDRD: 13-ERD-O42: 10/1/12-9/30/15 closed
LLNL/DOE $300,000
Optimizing Drug Efficacy
Role: Co-Investigator
The goal of this project is to develop novel methodologies for correlating genetic variation in humans with drug response. Understanding how the genetic make-up of a person influences drug efficacy and toxicity is key to developing customized effective treatments and minimizing harmful side effects.

Ognibene, Ted

Principal Investigator: Turteltaub, Kenneth W.

Active

P41GM103483 (Turteltaub) 6/1/2014 – 5/31/2019 1.8 Cal mo.
NIH/NIGMS $1,416,000

Resource for Development of Biomedical Accelerator Mass Spectrometry
The major goals of this project are to expand the present capabilities of BioAMS by developing a fully integrated HPLC/AMS interface, to study biochemical pathways down to the single cell level, and to validate methods for AMS in human translational research.
Role: Project Investigator

R21GM111242 (Turteltaub) 9/22/2014 – 6/30/2017 2.4 Cal mo.
NIH/NIGMS

Development of Laser Spectroscopic Methods For Quantification of ^{14}C
The goal of this project is to develop a spectroscopic instrument to quantify ^{14}C in biological samples.
Role: Project Investigator

30990 (Bench) 10/1/2014-09/30/2015 4.2 Cal mo.
LLNL
CAMS Biocarbon Operation
This project supports the operation of 1-MV BioAMS and 250 keV SSAMS Spectrometers
Role: Co-PI

39590 (Shaw) 10/1/2013-09/30/2016 3.6 Cal mo.
LLNL
PLS-Revolutionizing AMS
The major goal of this project is to ensure the installation of the new AMS system at LLNL along with its associated interfaces. Other goals include the adaption of the moving wire interface/gas ion source for carbon cycle research.
Role: Project Investigator

OVERLAP
None

Stewart, Benjamin

ACTIVE
P41GM103483 (Turteltaub) 6/1/14- 5/31/19 1.2 Cal mo.
NIH/NIGMS $1,466,000
Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)

The major goals of this project are to expand the present capabilities of BioAMS by developing a fully integrated HPLC/AMS interface, to study biochemical pathways down to the singe cell level, and to validate methods for AMS in human translational research.
Role: Project Investigator

US Department of Energy /LLNL 10/01/2012-present 8.4 Cal mo.
Projects supporting the strategic goals of US DOE.
Role: Research Scientist

*LLNL LDRD 6/01/2014 – 09/30/2016 2.4 Cal mo.
***In silico* system biology analyses of microbial communities**
The major goals of this project are to generate data to devlop and improve multiobjective flux models for biofuel production by microbial communities.

Principal Investigator: Turteltaub, Kenneth W.
Role: Research Scientist

OVERLAP
None

E. OVERALL IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

The Resource was provided funding to purchase two new analytical instruments: a Waters Xevo G2 XS QTOF instrument for qualitative and quantitative sample analysis and an Elementar BiovisION IRMS instrument for quantifying 13C/12C isotope ratios as well as CHN elemental analysis. The QTOF mass spectrometer provides sample characterization capabilities supporting AMS experiments. The QTOF enables accurate mass analyses for a variety of analytical applications, including metabolite profiling, identification, characterization, and quantification of both small and macromolecules. This instrument is capable of measuring ions with mass to charge ratios (m/z) ranging from approximately 150-20,000 and fills an analytical need not previously met with existing Resource equipment. The new QTOF is coupled to the Waters Acquity H Class HPLC system currently used to separate samples for introduction to the liquid sample AMS interface. A flow splitter is configured such that eluent from the HPLC flows to the QTOF for qualitative and quantitative analysis, and to the AMS instrument for isotope ratio measurement. Mass spectra are aligned based on retention times to match AMS results to mass spectrometric analytes. Coupling QTOF and AMS measurements in this way constitutes a powerful addition to our Resources analytical capabilities, and will significantly reduce sample analysis times since qualitative characterization of analytes will occur simultaneously with AMS measurements. The Elementar BiovisION IRMS instrument provides sample characterization capabilities supporting AMS experiments. This instrument allows quantitative information to be gathered from dual labeled species. This enhanced identification capability will enhance the analytical flexibility of the BioAMS laboratory. Both of these instruments are housed in the newly remodeled laboratory that houses the new BioAMS spectrometer. Together, the addition of these two instruments increases the analytical capabilities of the laboratory and the breadth of information we can obtain from our samples.

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. OVERALL CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

Funding for year 2 was below request so funds were reallocated within the resource to advance projects actively dedicated to using the technology development priorities of the research cycle. Active projects that utilized the HPLC-liquid sample interface were first to receive attention. Projects that utilization of the flow splitter to obtain simultaneous MA and AMS spectra are research priorities moving ahead.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

File uploaded: Human Subjects.pdf

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

Human Subjects

Summary of change: At the time of the grant submission, the Resource had one collaborative study that required the LLNL IRB full board review. That study has since been completed. At this time, all of the human samples that we analyze are de-identified or are "surgical excess" and are considered "exempt" under exemption number 4.

This proposal covers a technology development grant and does not involve direct oversight of any human subjects in its fundamental development projects. As a Research Resource, however, collaborators may involve human subjects in their research. The Resource is specifically aimed at higher sensitivity quantitation of biochemical interactions within living systems, and in fact encourages the use of AMS for quantifying very low chemical and radioisotope doses in humans of all ages.

The Resource requires that all proposed internal, collaborative and service projects involving humans must conform to NIH guidelines on the use of humans in medical research. All projects proposing the use of humans are required to provide evidence of an appropriate Institutional review and approval of all human protocols by Institutional Review Boards (IRB) of all institutions involved. After approval by the investigator's institution, the protocols are reviewed by LLNL's IRB and any suggested modifications are communicated to the institutions involved for rectification prior to final approval. No work begins at LLNL until LLNL's IRB approves the protocol or issues an exemption.

Protection of Human Subjects

All Resource activities that involve human subjects take place at collaborating performance sites. Resource personnel may be involved in the design of experiments to insure that the parameter of interest can be properly quantified by our technology. The Resource does not participate in the selection of human subjects but may inquire about the gender, age, physical size and physiological or medical status of the expected population in order to incorporate relevant information into the experimental design. The Resource encourages the use of the lowest possible chemical and radioisotope doses that still provide robust data across the expected population for the period of the experiment. These doses are inevitably much lower than are possible using any other analytical method. Most radiation exposures to human volunteers deliver effective doses integrated over their lifetimes (>50 years) equivalent to that incurred by a commercial airplane flight of a couple hours at altitude. Since such flights are apparently a commonly accepted risk in the general population, we advise our clients that there are no *a priori* reasons to exclude women of child-bearing age or even children from volunteer populations on radiation exposure grounds.

AMS is sensitive enough to detect the isotopic signals in microgram to milligram samples. These samples can be obtained from only a few microliters of blood or

very tiny (needle) biopsies. These samples, perhaps pre-fractionated by the collaborating research group, are sent to the Resource with no identifying information that allows Resource personnel to determine the personal source of the sample. A coded sample identifier having significance only to the collaborating scientist is required for sample tracking and reporting.

Potential risks to volunteers depend on the source and collection of the samples. The Resource advises against obtaining samples that are any larger than needed for the planned analyses.

Adequacy of Protection

The collaborative site recruits all volunteers and Resource personnel have no involvement in this process. Informed consent is evidenced by a sample Consent Letter that is required from the collaborating institution's stamped and approved IRB packet and is reviewed by the LLNL IRB. The LLNL IRB also reviews all advertising circulars and interview forms.

Personal information is kept at the institution hosting the human subjects and de-identified samples are provided to LLNL to protect confidentiality of personal information. All analyses currently performed by the Resource have been determined to be "Exempt" by the LLNL IRB.

G. OVERALL SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

Yes

Exemption number(s) E4

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional	Address

		District	
Primary: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB	827171463		LAWRENCE LVRMR NAT LAB 7000 EAST AVE LIVERMORE CA 945509234
LAWRENCE LIVERMORE NATIONAL LABORATORY	827171463		LAWRENCE LVRMR NAT LAB 7000 EAST AVE LIVERMORE CA 945509698
UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB	827171463		LAWRENCE LVRMR NAT LAB 7000 EAST AVE LIVERMORE CA 945509234
LAWRENCE LIVERMORE NATIONAL LABORATORY	827171463		LAWRENCE LVRMR NAT LAB 7000 EAST AVE LIVERMORE CA 945509698
UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB	827171463		LAWRENCE LVRMR NAT LAB 7000 EAST AVE LIVERMORE CA 945509234
LAWRENCE LIVERMORE NATIONAL LABORATORY	827171463		LAWRENCE LVRMR NAT LAB 7000 EAST AVE LIVERMORE CA 945509698
UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB	827171463		LAWRENCE LVRMR NAT LAB 7000 EAST AVE LIVERMORE CA 945509234

G.9 FOREIGN COMPONENT

Organization Name: University of Leicester
Country: UNITED KINGDOM
Description of Foreign Component:
Collaboration with Prof. Karen Brown at he University of Leicester, England. This collaboration is expected to result in co-authored manuscripts.

Organization Name: Karolinska Institute
Country: SWEDEN
Description of Foreign Component:
Collaboration with Prof. Kirsty Spalding at the Karolinska Institute in Stockholm, Sweden. This collaboration is expected to result in co-authored manuscripts.

Organization Name: St. Michael's Hospital
Country: CANADA
Description of Foreign Component:
Collaboration is with Dr. R. Loch Macdonald at St. Michael's Hospital in Toronto, Canada. This collaboration is expected to result in co-authored manuscripts.

Organization Name: Heinrich-Heine University
Country: GERMANY
Description of Foreign Component:
Collaboration is with Dr. Nima Etminan at Heinrich-Heine University in Dusseldorf, Germany. This collaboration is expected to result in co-authored manuscripts.

Organization Name: University of Heidelberg
Country: GERMANY
Description of Foreign Component:
Collaboration is with Dr. Nima Etminan at University of Heidelberg in Mannheim, Germany. This collaboration is expected to result in co-authored manuscripts

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Not Applicable

Composite Application Budget Summary

Categories	Budget Period
Salary, Wages and Fringe Benefits	434,849
Equipment	0
Travel	10,100
Participant/Trainee Support Costs	0
Other Direct Costs (excluding Consortium)	376,528
Consortium Costs	0
Direct Costs	821,477
Indirect Costs	644,870
Total Direct and Indirect Costs	1,466,347

Component Budget Summary

Components	Categories	Budget Period
8634-001 (Admin Core)	Salary, Wages and Fringe Benefits	434,849
	Equipment	0
	Travel	10,100
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	376,528
	Consortium Costs	0
	Direct Costs	821,477
	Indirect Costs	644,870
TOTALS	Total Direct and Indirect Costs	1,466,347
8621-001 (Core)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
8622-002 (Core)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0

	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
8623-003 (Core)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
8633-004 (Core)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0

8631-005 (Core)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
8624-001 (Project)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
8625-002 (Project)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0

	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
8626-003 (Project)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
8627-004 (Project)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
8628-005 (Project)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0

	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
8629-006 (Project)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
8630-007 (Project)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0

8632-001 (Training)	Salary, Wages and Fringe Benefits	0
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	0
	Consortium Costs	0
	Direct Costs	0
	Indirect Costs	0
TOTALS	Total Direct and Indirect Costs	0
TOTALS		**1,466,347**

Categories Budget Summary

Categories	Components	Budget Period
R&R Budget - Senior/Key Person Funds Requested	8634-001 (Admin Core)	338,455
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		338,455
R&R Budget - Other Personnel Funds Requested	8634-001 (Admin Core)	96,394
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0

	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		96,394
R&R Budget - Section A & B. Total Salary, Wages and Fringe Benefits (A+B)	8634-001 (Admin Core)	434,849
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0

TOTALS		434,849
R&R Budget - Section C. Total Equipment	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Domestic Travel	8634-001 (Admin Core)	10,100
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0

	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		10,100
R&R Budget - Foreign Travel	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Section D. Total Travel	8634-001 (Admin Core)	10,100
	8621-001 (Core)	0

	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		10,100
R&R Budget - Tuition/Fees/Health Insurance	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0

	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Stipends	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Trainee Travel	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0

	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Subsistence	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0

TOTALS		0
R&R Budget - Other Participants/Trainee Support Costs	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Section E. Total Participants/Trainee Support Costs	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0

	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Materials and Supplies	8634-001 (Admin Core)	94,738
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		94,738
R&R Budget - Publication Costs	8634-001 (Admin Core)	0
	8621-001 (Core)	0

	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Consultant Services	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0

	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - ADP/Computer Services	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Subawards/Consortium/Contractual Costs	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0

	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Equipment or Facility Rental User Fees	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0

TOTALS		0
R&R Budget - Alterations and Renovations	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Other Direct Cost 1	8634-001 (Admin Core)	281,790
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0

	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		281,790
R&R Budget - Other Direct Cost 2	8634-001 (Admin Core)	0
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Other Direct Cost 3	8634-001 (Admin Core)	0
	8621-001 (Core)	0

	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		0
R&R Budget - Section F. Total Other Direct Cost	8634-001 (Admin Core)	376,528
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0

	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		376,528
R&R Budget - Section G. Total Direct Cost (A thru F)	8634-001 (Admin Core)	821,477
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		821,477
R&R Budget - Section H. Indirect Costs	8634-001 (Admin Core)	644,870
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0

	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0
TOTALS		644,870
R&R Budget - Section I. Total Direct and Indirect Costs (G +H)	8634-001 (Admin Core)	1,466,347
	8621-001 (Core)	0
	8622-002 (Core)	0
	8623-003 (Core)	0
	8633-004 (Core)	0
	8631-005 (Core)	0
	8624-001 (Project)	0
	8625-002 (Project)	0
	8626-003 (Project)	0
	8627-004 (Project)	0
	8628-005 (Project)	0
	8629-006 (Project)	0
	8630-007 (Project)	0
	8632-001 (Training)	0

TOTALS		1,466,347

A. COMPONENT COVER PAGE

Project Title: TR&D1 Quantitative Protein Biochemistry and Macromolecular Turnover
Component Project Lead Information: Stewart, Benjamin J

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Scope: To develop methods for macromolecule labeling, characterization and quantitation enabling cell and tissue turnover studies and absolute quantitation of natural and synthetic macromolecules and their modifications in important biological systems.
Since its inception in 1999, the NIH-funded Research Resource on the biomedical applications of accelerator mass spectrometry (AMS) has performed numerous groundbreaking studies in many areas including biomolecule turnover, quantitative metabolism of endogenous compounds and xenobiotics, and analysis of potentially mutagenic adducts. These studies were made possible by the high sensitivity, accuracy, and precision of AMS. The work proposed in this TR&D core will build on this legacy of successful, high-impact research and will extend the applicability of current AMS experimental and analytical methods to a wider set of scientific and technical problems. The focus for this core is the development and routine implementation of methods for absolute quantitation of proteins and other biological macromolecules. This core will support several driving biomedical projects, including: 1) Characterization of turnover rates of tissues and cell types labeled as a result of anthropogenic atmospheric 14C release (bomb-pulse biology); 2) Absolute quantitation of protein post-translational modifications; 3) Identification of receptors based on 14C-ligand binding; and 4) Development of labeling strategies, separation techniques, and analytical procedures for quantitation of computationally-designed synthetic protein drugs in biological matrices, in support of animal dosing experiments and human microdosing studies.
Biological macromolecules play a wide variety of essential roles in cells, tissues, and organisms. Proteins are the basic building blocks of all living organisms, and proteinaceous enzymes catalyze the chemical reactions responsible for life. Nucleic acids provide the genetic material containing information for building, maintaining, and regulating living organisms. Because biological macromolecules play such essential and diverse roles, improvements in our ability to quantitatively trace macromolecules and their modifications will enable important discoveries that will significantly advance our understanding of biological processes. In a recent paper, Hanke et al (Hanke et al., 2008) wrote:
“Ultimately, it would be highly desirable to obtain exact quantitative values of each protein in a system, e.g., their copy number per cell or their concentration in nanogram per milliliter of body fluid. While this kind of basic information about a protein is already per se valuable for the biologist, systems biology even requires it as input for modeling. In a medical context, knowing the exact amounts of certain proteins in blood or other common sources of biomarkers can provide diagnostically relevant information for patient treatment.”
Analytical measurement of proteins and other biological macromolecules presents a number of significant technical challenges, particularly when quantitation is required. Macromolecule extraction from biological matrices is typically more complex than the extraction of small molecules, and extracts are more prone to contamination. Macromolecules such as proteins and nucleic acids are heterogeneous molecules, and may be chemically modified as a result of enzyme-mediated reactions (e.g., post-translational modifications) or as a result of tissue processing, as in the case of formalin-fixed tissue. In addition, biological macromolecules may be susceptible to cleavage by physical or chemical means. While analytical standards can usually be purchased or synthesized for small molecules of interest, similar standards are rarely available for biological macromolecules. The ability to measure absolute quantities of biological macromolecules has broad relevance for biology and medicine, as there currently is no standardized, universal analytical method capable of making these measurements. The major focus of this core is to develop methods for applying AMS technology, especially in conjunction with the new liquid sample AMS interface, to overcome these major challenges of biological macromolecule analysis. To this end, we propose to interact with collaborating researchers to solve a variety of important biological problems.
Specific Aim 1: Improve isolation and characterization techniques to determine macromolecular age in specific cell types. Cell turnover studies provide important information about basic biological processes and have important therapeutic implications. However, these studies are limited by the need for large quantities of precious material. Additionally, only samples that have been prepared in a manner compatible with AMS analysis can be measured. This limitation currently prevents the analysis of most archived tissue samples. This specific aim therefore proposes to improve separation protocols to utilize reduced sample sizes for bomb pulse macromolecular turnover studies and attempt the development of methods for macromolecule isolation from archived tissue samples.
Specific Aim 2: Develop methods for protein post-translational modification (PTM) labeling to enable absolute quantitation of PTMs in cultured cells. Post-translational modifications of proteins are important regulators of protein function and longevity, with major impacts on cell biology. Relative quantitation of protein PTMs is commonly accomplished using stable isotope tags, and provides important information about protein function. However, absolute quantitation of PTMs remains elusive using stable isotopes and is essential for quantitation of PTMs located on overlapping peptides. Characterization of combinatorial protein modifications is necessary to advance our understanding of how PTMs interact to control protein function. This aim proposes to develop protocols for incorporation of 14C-PTMs into proteins of interest, enabling absolute quantitation of PTMs in intact proteins and in peptides by making use of the new AMS-liquid sample interface.
Specific Aim 3: Develop methods for receptor identification based on binding of 14C-labeled ligands. Knowledge of receptor-ligand interactions is critical for a detailed understanding of fundamental biology, and is an important basis for devising new pharmaceutical strategies to treat diseases. Identification of receptors for compounds of biological importance is a difficult task due to challenges in isolating the receptor-ligand complex and demonstrating binding specificity. This aim seeks to develop methods for using highly-specific 14C-labeled ligands to identify ligand receptors, and will develop new labeling strategies and peptide separation protocols to exploit the liquid sample interface for high-throughput, rapid screening of ligand-receptor interactions. Specific Aim 4: Develop label incorporation strategies and separation protocols to enable 14C-AMS tracer studies with synthetic therapeutic proteins and peptides. Protein therapeutics and other biologicals are increasingly important in medicine. Unfortunately, methods for tracing biologicals in vivo are limited by poor sensitivity and specificity. Improved methods for synthetic therapeutic proteins will enable detailed pharmacokinetic and pharmacology studies on these compounds, reducing drug development time and associated costs. This specific aim proposes to develop appropriate methods for labeling therapeutic proteins with 14C without altering structural properties and desired biological activity, and to develop methods for measurement of 14C-labeled proteins in relevant biological matrices using the AMS liquid sample interface.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: TR&D1 progress_2016.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Results were disseminated in publications and presentations. Alterations in tryptophan metabolism in renal cell carcinoma were reported in the journal Cancer Research. Critical Role of Histone Turnover in Neuronal Transcription and Plasticity (Maze et al, 2015) extended bomb pulse dating to a specific isolated and purified histone protein. Dr. Nima Etminan (CP3) presented several talks on turnover of structural proteins of aneurysms and cerebral arteries and the clinical implications.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Specific Aim 1.1 (Develop protocol to separate major lipid classes for bomb pulse dating) and Specific Aim 1.3 (Harvest the structural collagen in the extracellular matrix of adipose tissue and measure its turnover) will be addressed in the next reporting period. Both of these aims are in support of DBP1 which is on hold while PI Kirsty Spalding is on personal leave. Specific Aim 1.4 (Develop protocol to quantitatively extract DNA from archived FFPE specimens for bomb pulse dating) is on hold pending funding. A proposal was resubmitted by Donald Vander Griend (University of Chicago) in October 2015 and scored in January 2016. Interest in turnover of extracellular matrix (structural proteins) has been expressed by several researchers not yet collaborating in the Resource and scoping studies are planned for summer 2016.

Specific Aim 2.1 (Test chemical 14C-PTM labeling techniques in model cell systems and characterize labeling efficiency) and Specific Aim 2.2 (Develop and refine labeling procedures for 14C-AMS measurement of PTMs using metabolic precursors based on published levels of incorporation and additional mass balance experiments). Due to budget limitations, these experiments have been scaled down. Experiments will be performed to generate and characterize chemical adducts on proteins and DNA generated using 14C-labeled precursors. Initial experiments will be performed in vitro using model proteins or DNA. These adducts will be used to demonstrate the utility of the combined LC-MS-AMS measurement platform and to determine and report measurement parameters for labeled macromolecules. These conditions can then be used to measure protein post-translational modifications in subsequent experiments.

Specific Aim 2.2 (Develop and refine labeling procedures for 14C-AMS measurement of PTMs using metabolic precursors based on published levels of incorporation and additional mass balance experiments). Experiments will be performed using 14C-labeled glucose in cell culture media to label histone modifications using the yeast S. cerevisiae as a model system. Label levels will be optimized to ensure adequate signal for LC-MS and AMS detection of histone modifications. Peptide analysis experiments will be performed using the combined LC-MS-AMS platform. Total label incorporation into proteins and PTMs will be determined.

Specific Aim 4.2.1: Develop methods for extraction of 14C-labeled synthetic proteins from biological matrices. We will characterize binding of biotinylated 14C-methylated sclerostin antibody to sclerostin in vitro using an immunoprecipitation assay combined with LC-MS-AMS. Binding will be quantified to determine whether binding specificity is altered. Labeled, biotinylated antibody will be characterized for in vivo administration to rodents.

Though not listed as a specific aim, we plan to continue using the system to measure naphthalene adducts of DNA from mouse lung tissue.

Specific Aim 1: Improve isolation and characterization techniques to determine macromolecular age in specific cell types.

While acquiring data for CP3A on collagen dating of aneurysms (Etminan et al, 2014) it became clear that normal arteries sought as controls have very low collagen content, so little that it was not possible to make viable samples for AMS analysis. Initial attempts by CP3B to isolate collagen from arteriovenous malformations (AVMs) likewise produced very low yields of collagen. A procedure to isolate pure elastin from cerebral arteries and AVMs was developed in collaboration with both CP3 groups. The elastin from approximately 85 arteries and 15 AVMs has been dated. The data is being used to construct a model of elastin growth and turnover throughout life in normal cerebral and internal carotid arteries and is being compared turnover in AVMs. The paper reporting the results is in early draft and will be submitted during the next reporting period.

A procedure for acquiring and purifying zonules for bomb-pulse dating is also underway in collaboration with Prof. Stephen Bassnett of Washington University. Zonules are a series of fibers connecting the ciliary body and lens of the eye, holding the lens in place.

Additionally, we are working on developing high purity RNA separations free of carrier carbon suitable for dating food sources for cells and tracing lablelled components with the liquid sample interface.

Specific Aim 1.4: Develop protocol to quantitatively extract DNA from archived FFPE specimens for bomb pulse dating.

We assisted Donald VanderGriend in preparation of a R21 proposal submitted fall 2015 to provide substantial support for this aim beyond the exploratory stage. The proposal was scored and has proceeded to council.

Specific Aim 3: Develop methods for receptor identification based on binding of 14C-labeled ligands.

Specific Aim 3.1: Rapidly screen cell extracts for high 14C-ligand binding

This aim develops methods to rapidly screen proteins and peptides for bound excess ^{14}C-ligand (the epoxyeicosatrienoic acids (EETs)) to identify receptor(s). Using 14C-EET synthesized at UC Davis, the Hammock group identified primary human coronary artery smooth muscle cell as the model cell line for investigation.

Specific Aim 4.1: Develop and validate methods for ^{14}C-labeling of synthetic therapeutic proteins for administration to animals in pharmacokinetic studies.

Specific Aim 4.1.1: Develop chemical labeling methods for introduction of ^{14}C label into synthetic proteins to enable AMS absolute quantitation.

In support of DBP4, an antibody to the protein sclerostin was biotinylated and reductively methylated in preparation for administration to animals. Additional protein characterization is currently in progress.

Specific Aim 4.1.2: Develop methods for metabolic ^{14}C-labeling of synthetic proteins for use in animal biodistribution experiments. Metabolic labeling experiments were performed with ^{14}C-glutamine and ^{14}C-tryptophan. The primary focus was on metabolites of glutamine and tryptophan in renal cell

carcinoma and normal cell lines. The bulk of ^{14}C label was incorporated into cellular macromolecules (proteins) that were not fully characterized. Additional metabolic labeling experiments are not planned at this time since chemical labeling methods are currently more versatile and appear to provide satisfactory labeling.

Although not a specific aim in the original proposal, effort in this core was directed to the installation of a Waters Xevo G2-XS QTOF mass spectrometer in the Bio-AMS laboratory. The instrument was coupled to the AMS liquid sample interface using a flow splitter. A custom device was designed and built to enhance readout accuracy of the flow splitter, enabling reproducible flow splitting from the HPLC to the mass spectrometer and the AMS liquid sample interface. Using this coupled system, ^{14}C-tryptophan metabolites were separated and analyzed by HPLC, mass spectrometry, and AMS. The system was also used to measure naphthalene adducts of DNA from mouse lung tissue.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

A modified method for analysis of tryptophan pathway metabolites by HPLC was developed and a methods paper is in preparation for submission and review to the journal Bio-Protocol.
The procedure for isolating ultra pure elastin from arteries was developed and will be published during the next reporting period.

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?
Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?
Not Applicable
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?
NOTHING TO REPORT
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B DRAFT

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: TR&D2 Quantitative Endpoints in Humans
Component Project Lead Information: Michael, Malfatti

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

A major goal of this project is to develop robust extraction and HPLC/UPLC separation methods of metabolites, macromolecules, and synthetic proteins to make them amenable for on-line LS-AMS quantification. This will enable the use of smaller sample sizes and lower specific activity requirements leading to safer and less invasive procedures for human sample collection. The specificity and sensitivity afforded by LS-AMS will enable highly specific and reproducible assays to be developed for quantification of a variety of human endpoints in response to very low doses of chemicals/drugs. Although the tools we develop will be applicable to many types of xenobiotics and endogenous compounds, we intend to focus on the pharmacokinetics (PK) and biodistribution of radiocarbon-labeled synthetic protein therapeutic lead compounds, environmentally prevalent aromatic hydrocarbons, chemotherapy drug-DNA adducts, and 14C-thymidine-labeled cancer cells. These projects were chosen due to a combination of unmet medical need, technical feasibility, input from collaborators and the potential to engage the larger NIH-funded community upon success. To accomplish these goals the following specific aims are proposed.
Specific Aim 1: Develop techniques for the isolation and extraction of 14C-labeled synthetic therapeutic proteins from low dose PD/PK studies for liquid sample AMS compatibility (this specific aim interacts closely with specific aim 4.2 of core TR&D 1).Protein-based therapeutics possess great potential in treating many types of diseases. However, a major challenge that limits their use is the lack of quantitative determination of the proteins biological fate due to lack of stability and short half-lives. A thorough understanding of therapeutic protein biodistribution, metabolism, and pharmacokinetic characteristics is vital for biologic development and assessment of efficacy and safety. To accurately quantify the pharmacokinetics (PK) and biodistribution of synthetic proteins we will develop methods to isolate 14C-labeled proteins from biological matrices after IV or oral exposures to rodents to make them compatible with LS-AMS without compromising the integrity of the protein.
Specific Aim 2: Develop high efficiency sample processing methods for the extraction and separation of highly toxic or carcinogenic compounds and their metabolites from human samples derived from environmentally relevant human exposures.Polyaromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants and are of potential concern to human health. There is little information on the pharmacokinetics of PAHs in humans. Given the potential toxicity of PAHs and their metabolites, doses to human subjects must be kept low to minimize the possibility of study-related adverse health effects. This constraint results in a low measurable signal, even with the sensitivity of AMS. We propose to develop highly efficient LS-AMS compatible extraction and sample concentration procedures to improve the signal-to-background ratio. Specifically, we will optimize extraction procedures and Ultra Performance Liquid Chromatography (UPLC) conditions for the separation of PAHs and their metabolites from human urine and plasma from dose-limited exposure studies for quantitation of PK parameters by LS-AMS analysis.
Specific Aim 3: Develop sample processing methods for LS-AMS analysis, to support feasibility studies of microdose-based prediction of chemotherapy efficacy.Microdose-induced platinum-based drug-DNA adducts, as measured by AMS, are emerging as clinically useful biomarkers of chemotherapy efficacy. There are currently two clinical studies accruing patients that are using 14C-carboplatin or 14C-oxaliplatin as chemical probes of the capacity of tumors to take up these drugs and form drug-DNA damage. Since these agents are nearly always used in combination with another drug, it is desirable to develop dual radiocarbon dosing and sample analysis capabilities. This Aim will pursue the adoption of LS-AMS to the ongoing clinical program and development of a protocol to measure simultaneously the drug-DNA adducts formed from microdoses of 14C-carboplatin and 14C-gemcitabine administered to mice bearing patient-derived tumor xenografts.
Specific Aim 4: Optimize 14C labeling strategies and DNA isolation methods for quantifying the metastatic potential of single tumor cells that are highly labeled with 14C-2'-deoxythymidine in a mouse tumor xenograft model.The inability to effectively treat metastatic disease is the main reason for the limited progress in reducing the rates of cancer morbidity and mortality. One major drawback is the lack of understanding of the metastatic process, in which it is assumed, but not proven, that a single cell migrates to a new site and seeds the growth of a new tumor. In this aim we will develop and optimize radiocarbon labeling and detection strategies for 14C- thymidine incorporation into the DNA of prostate cancer cells. This will enable quantification of microscopic tumor initiation across an entire mouse. The labeling optimization will aim to achieve sufficient signal such that a tumor derived from a single cell can be detected by LS-AMS.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: TR&D2_accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: TR&D2 training.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Results have been disseminated through publications and presentation of results at scientific conferences and meetings. See overall dissemination list.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

In the coming year we will continue to optimize methods for extracting ultra-low concentrations of 14C-labeled compounds from human samples for pharmacokinetic and metabolite analysis. The goal is to increase the extraction efficiency while maintaining compatibility for AMS quantification via the liquid sample interface. We will also continue with UPLC methods development for separation and isolation of metabolites while maintaining compatability (solvent, flow rate and sample volume requirements) with the AMS moving wire interface

We will also optimize sample preparation of neat biological samples (i.e. plasma, homogenized tissue) for single drop analysis using the moving wire interface and AMS quanitifacation.

To support DBPs we will develop robust sample processing methods to allow for DNA adduct quantification from small human tissue and/or blood samples by AMS with an emphasis on reducing signal-to-noise. We will also continue to develop extraction procedures for sample compatibility for liquid sample AMS analysis.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

A major goal of this project is to develop robust extraction and HPLC/UPLC separation methods of metabolites, macromolecules, and synthetic proteins to make them amenable for on-line LS-AMS quantification. This will enable the use of smaller sample sizes and lower specific activity requirements leading to safer and less invasive procedures for human sample collection. The specificity and sensitivity afforded by LS-AMS will enable highly specific and reproducible assays to be developed for quantification of a variety of human endpoints in response to very low doses of chemicals/drugs. We are focusing our efforts on the pharmacokinetics (PK) and biodistribution of radiocarbon-labeled synthetic protein therapeutic lead compounds, environmentally prevalent aromatic hydrocarbons, chemotherapy drug-DNA adducts, and ^{14}C-thymidine-labeled cancer cells.

Specific Aim 1: Develop techniques for the isolation and extraction of ^{14}C-labeled synthetic therapeutic proteins from low dose PD/PK studies for liquid sample AMS compatibility.
Nothing to report

Specific Aim 2: Develop high efficiency sample processing methods for the extraction and separation of highly toxic or carcinogenic compounds and their metabolites from human samples derived from environmentally relevant human exposures.
Methods have been developed to extract low concentrations of ^{14}C-PAHs from human plasma and urine samples. The extraction efficiency was approximately 55% recovery of the PAH. Further testing revealed that reconstitution of the compound with toluene resulted in an increase in extraction efficiency to 89%. Extraction methods have been developed for efficient extraction of PAHs and metabolites from the biological matrix. Chromatographic methods development is underway for the separation and isolation of PAHS and metabolites using UPLC techniques. For compatibility with the moving-wire AMS system, flow rate, solvent compatibility, and sample volume need to be considered. Preliminary samples have been quantified by AMS using the moving-wire interface.

Specific Aim 3: Develop sample processing methods for LS-AMS analysis, to support feasibility studies of microdose-based prediction of chemotherapy efficacy.
In conjunction with DBP 6, Standards were developed for DNA containing carboplatin-DNA adducts which range from concentrations that can be detected by liquid scintillation counting (LSC) down to those that can be quantified by AMS that cover the range expected for patient samples. These standards were measured on three AMS instruments and there was no statistically significant differences in any of the measurements. This is good proof-of-concept for (1) widespread use of AMS as a diagnostic tool for patients and (2) the LLNL moving wire system. These standards will enable meaningful comparisons between graphite and liquid samples in order to evaluate the performance of the moving wire AMS system compared to two other well-established systems (AMS of graphite and decay counting by LSC). Mice containing four different human-derived tumors were given microdoses and therapeutic doses of [^{14}C]carboplatin and [^{14}C]gemcitabine, which were measured by AMS, and the results compared to tumor response to full does cisplatin- or carboplatin-gemcitabine therapy. The exposure of two labeled drugs has enabled the development of sample preparation techniques to analyze 2 different drug DNA adducts by AMS from a single sample. The optimization of these methods are continuing.

Specific Aim 4: Optimize ^{14}C labeling strategies and DNA isolation methods for quantifying the metastatic potential of single tumor cells that are highly labeled with ^{14}C-2'-deoxythymidine in a mouse tumor xenograft model.

The inability to effectively treat metastatic disease is the main reason for the limited progress in reducing the rates of cancer morbidity and mortality. One major drawback is the lack of understanding of the metastatic process, in which it is assumed, but not proven, that a single cell migrates to a new site and seeds the growth of a new tumor. In this aim we will develop and optimize radiocarbon labeling and detection strategies for ^{14}C- thymidine incorporation into the DNA of prostate cancer cells. This will enable quantification of microscopic tumor initiation across an entire mouse. The labeling optimization will aim to achieve sufficient signal such that a tumor derived from a single cell can be detected by LS-AMS.

Using a highly metastatic prostate cancer cell line (PC3), cancer cells were labeled with C14TdR in tissue culture. Labeling conditions have been optimized so that the detection of ^{14}C-thymidine incorporation in to the DNA could by quantified by AMS from a single cell. The Labeled PC3 cells were delivered into NSG mice and tracked in vivo. Recovered cells were quantified by AMS to measure the degree of metastasis. Sample preparation and extraction methods have been developed to isolate and quantify the ultra low levels of 14C-thymidine incorporation from the prostate tumor cell DNA derived from the ^{14}C-labeled prostate cancer cells.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

TR&D2 Training

Hosted two student interns between 06/2015 -08/2015.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

Yes

Exemption number(s) E4

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE Not Applicable
G.11 PROGRAM INCOME Not Applicable
G.12 F&A COSTS Not Applicable

RPPR - Core-8622

RESEARCH & RELATED BUDGET - SECTION A & B **DRAFT**

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									**Total Senior/Key Person**	

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: TR&D3 Liquid Sample AMS
Component Project Lead Information: Ognibene, Ted

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The work proposed in this TR&D will increase the accessibility and simplicity of AMS by moving the spectrometer away from highly specialized facilities along with the reduction of the unique complexities of AMS sample preparation. This TR&D will validate a new high voltage deck AMS system with a gas-accepting ion source for use in a biochemical laboratory and develop the routine use of directly coupled HPLC-AMS quantitation of biochemicals. This effort is a natural progression of our work conducted over the past four years where we have developed a liquid sample interface that converts the carbon content in biomedical samples, dissolved or suspended in liquids, to CO2 for introduction directly into the gas-accepting ion source for real-time AMS analysis and the acquisition of a National Electrostatics Corporation 250kV accelerator mass spectrometer that will be situated in a biochemical laboratory.

While high voltage decks have been validated for use with samples introduced as graphite and are in use in a number of AMS laboratories, we aim to demonstrate and validate the use of a high voltage deck for the analysis of biomedical samples introduced as CO2 gas, which has not been done previously and is essential to furthering the use of AMS in broad based biomedical research. In addition, the high voltage deck AMS system is to be located in a BSL-2 biological lab setting and will be developed for use by the non-specialist. We will increase our capabilities for analysis of speciated molecules by continuing development on our interface to enable the direct coupling of an HPLC to the gas accepting ion source.

A major focus will be to develop a fully integrated and robust measurement capability for HPLC which is the most common method used by our collaborators for speciation of cellular or tissue components. We will continue the development of an integrated HPLC-AMS system to provide the same robust measurement capabilities we now have in place with our solid sample analysis methods to speed throughput, reduce costs and enable AMS quantitation of complex mixture components for radiocarbon labeled molecules. This technology development will enable more rapid and cost effective HPLC analysis and lead to new directions for mechanistic studies. It will also increase the throughput and ease of use of AMS through direct coupling to separatory instruments and enable us and our collaborators to more readily obtain high-density data sets.

The Specific Aims along with the expected periods of activity of this TR&D are outlined as follows:
Specific Aim 1. Validation of new high voltage deck AMS system with a gas-accepting ion source for use in a biochemical laboratory.
Specific Aim 1.1. Determine sensitivity and dynamic range (Year 1).
Specific Aim 1.2. Construct and validate interface for use on the SSAMS (Years 1-2).
Specific Aim 2. Develop the routine use of 14C-AMS analysis from small liquid samples.
Specific Aim 2.1. Determine the 14C and 12C dynamic range (Years 1-2).
Specific Aim 2.2. Automation of liquid drop placement (Years 2-5).
Specific Aim 2.2.1. Establish automated liquid drop placement (Years 2-3).
Specific Aim 2.2.2. Enable multiple drop capability (Years 3-4).
Specific Aim 2.2.3. Enable automated sample analysis (Years 3-5).
Specific Aim 3. Develop the routine use of directly coupled HPLC-AMS quantitation of biochemicals.
Specific Aim 3.1. Examine the effect buffers and salts used in HPLC methods have on drying, combustion and background levels (Years 1-5).
Specific Aim 3.2. Minimize and control sample to sample memory (Years 1-4).
Specific Aim 3.3. Investigate different metals for use as CO2 adsorption and ionization in reducing memory (Years 4-5).

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: TR&D3_accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: TR&D3 training.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Dissemination of TR&D 3 during this preformance period consist of 3 peer-reviewed manuscripts and 1 invited presentation.

1). Madeen E, Corley RA, Crowell S, Turteltaub K, Ognibene T, Malfatti M, et al. (2015). Human in Vivo Pharmacokinetics of C-14 Dibenzo def,p chrysene by Accelerator Mass Spectrometry Following Oral Microdosing. Chemical Research in Toxicology 28: 126-134.

2). Ognibene TJ, Haack KW, Bench G, Brown TA, & Turteltaub KW (2015). Operation of the ,"Small" BioAMS spectrometers at CAMS: Past and future prospects. Nucl Instrum Meth B 361: 54-57.

3). Ognibene TJ, Thomas AT, Daley PF, Bench G, & Turteltaub KW (2015). An interface for the direct coupling of small liquid samples to AMS. Nucl Instrum Meth B 361: 173-177.

4). Ognibene TJ, Applications of Accelerator Mass Spectrometry to Human Health Research, presented at the Pittsburgh Conference on Analytical, Chemistry and Applied Spectroscopy (PITTCON 2016), Atlanta, GA, held March 3-11, 2016.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

During the next reporting period, we plan to continue with the characterization and validation of the moving wire interfaces with our new spectrometer. Specifically:
1)We plan to begin automation of liquid drop placement.
2)We plan to continue our validation of LS-AMS by measuring samples from different biological matrices and comparing those results to graphite samples.
3)We plan to continue our examinations of the effect that HPLC mobile phases have on the transfer, drying and combustion of separated biochemicals.
4)We plan to demonstrate LS-MS/AMS capability on experiments of biological relevance.

While the above activities are proceeding, we will continue to provide AMS analysis for our collaborations and DBPs of both solid and liquid samples.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

During this reporting period, we have made several modifications to the SSAMS to improve efficiency, accuracy and reliability in measured samples. We identified a problem with the data acquisition system as supplied by the manufacturer, NEC. The main issue was the digital signal processor (DSP) used to convert analog ion peak signals to digital waveforms for analysis was rejecting and not accounting for otherwise valid events. The net result was that for high-count rate samples, the measured 14C/13C isotope ratio was underestimated by up to 20% in some instances. We worked with software programmers at NEC and corrected the deficiencies in the DSP to reduce the ion rejection rate and to properly account for all "missing" 14C events. We have tested these corrections and find them accurate to better than 99.4% for 14C count rates up to 65 kHz, or approximately 250 Modern for a typical graphite sample.

We have completed our next phase in hardware modifications to the ion source region of both ion sources. We replaced the einzel lens housing with one that was longer and able to accommodate a 300 l/s turbo molecular vacuum pump. This additional pumping is required to remove neutral helium carrier gas that is introduced to the ion source along with the CO2 pulse from the moving wire interface tables.

We now realize 13C ion currents and 14C count rates that are on par, if not slightly higher, than what we had achieved with our 1-MV AMS system for graphite targets.

We have completed the installation of the moving wire interface tables and have begun validation testing. We demonstrated a positive comparison with graphite targets for aqueous sugar solutions with isotope ratios between 1 and 180 Modern. We are beginning comparisons with other sample matrices.

We purchased and coupled a Waters QTOF MS to our Waters H-Class UPLC, which is part of the moving wire interface. We designed a quantitative flow splitter to split the output of the UPLC between the MS and the AMS spectrometer, creating a unique LC-MS/AMS capability to provide both qualitative and quantitative data from LC-separated biomolecules.

TR&D3 Training

We provided one-on-one training in the operation of the moving wire interface to Resource staff and collaborators, conducting research in an NIEHS-funded study on the pharmacokinetics of PAHs in human volunteers. We have trained Resource staff on the operation of our new AMS spectrometer and moving wire interface operation. Such training entails the one-on-one mentoring on the procedures needed to successfully load samples into the ion source, turn on and tune the spectrometer, configure the data acquisition system to measure the samples to defined performance criteria.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Research activities funded through this Resource include the development of our Liquid Sample AMS (LS-AMS) measurement capabilities. LS-AMS enables the measurement of biochemicals containing a few 10s of nanograms carbon and as little as 50 zmol of 14C at a fraction of the cost and with a significant reduction in sample preparation time as compared to our traditional method of solid sample analysis. We share this technology through press releases, presentations at conferences and through visitors to the Resource. In addition, we sponser outside collaborators to come to LLNL and perform research using our technology.

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

File uploaded: TR&D3 C5 other products.pdf

C.5.b Resource sharing

NOTHING TO REPORT

TR&D 3

C.5 Other Products and Resource Sharing

We have developed a computer program for the analysis of liquid sample AMS data (*LS-AMS Analysis*). The standalone executable program runs on either OSX or Windows PC and is available for collaborators using our moving wire interface for the analysis of small liquid samples. The program is available to users of this Research Resource by contacting Ted Ognibene (resource staff member).

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Current AMS analysis using graphite requires samples with a carbon content of ~50 µg or greater regardless of the amount of 14C label in the sample. For our liquid sample interface, samples having a carbon content of 1 µg can readily be analyzed for 14C content at contemporary levels (14C/12C ratio of 10–12). For highly labeled material, sample sizes as small as a 1 picogram (pg) can be analyzed with the liquid sample interface. This extremely small sample size affords the important capability of readily quantifying low-abundance metabolites in metabolite profiles and improves on measurement accuracy because there is no need to add a carrier carbon-bulking agent that is often utilized to enable small-sample graphite AMS measurement.
Current AMS analysis of biological materials requires that carbonaceous biochemicals be converted to graphite. However, considerable human handling is required, and the process suffers from low sample throughput (<100 samples processed per day) and long turnaround times (~2 days) at a current cost of $120 per sample at LLNL. With our new method, the time required for analysis of biochemical samples by AMS is drastically reduced from days to minutes at a cost of less than $50 for a single sample. Since liquid separation technologies are universally used in biomedical applications to separate a mixture of compounds into individual components for further study an important feature is that the new method allows for continuous flows of liquid, enabling direct coupling of an HPLC or other liquid separation technology to the spectrometer for real-time AMS analysis. In performing a single HPLC-AMS trace using graphite samples, eluant from an HPLC trace is frequently collected in sixty 30-second fractions. It takes approximately two to three days to convert the fractions to graphite and then analyze them for 14C content. At a cost of $120 per sample, the total cost is $7,200 for a single HPLC–AMS trace. Many users find such costs prohibitive. In contrast, the liquid sample interface can take the output from an HPLC directly and perform 14C analysis online in the time it takes the HPLC to speciate the sample: in other words, a total of time of 30 minutes at a total cost of approximately $700 to $1,000.
Biomedical users have been slow to adopt AMS because of the high cost and complexity of sample preparation with graphite methodology and resulting slow turnaround times for analysis. Our new technology will enable the market to greatly expand by removing these important financial and temporal constraints. This development provides an integrated system and eliminates complex and time-consuming human handling of samples thus making the instrument much simpler to operate by general laboratory staff in clinical or biological laboratory settings.

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

Analyzed ion currents from the new spectrometer as delivered from the manufacturer were a factor of 3 lower than what we obtained from our 1-MV AMS system. Additionally, there were critical shortcomings with the data acquisition system that prevented the accurate measurement of high-count rate samples, forcing us to limit ion source output. Lower ion currents impacts sample measurement throughput for solid graphite samples and sensitivity for gaseous CO2 samples. We have completed steps to improve ion yield and transmission. We replaced the argon gas used in the molecular ion destruction cell with helium to improve transmission. We completed modifications to ion source electrodes and can now operate at higher cathode potentials with increases in ion yields to acceptable levels. We also engaged the manufacturer to correct the errors in the data acquistion system and can now accurately measure high-count rate samples.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-8623

RESEARCH & RELATED BUDGET - SECTION A & B **DRAFT**

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: DBP1 Bomb Pulse Biology

Component Project Lead Information:

Spalding, Kirsty

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

This DBP seeks to determine the carbon turnover of major lipid classes and select proteins associated with lipid tissue structure and lipid storage and regulation. This project will compare the differences in turnover of the target molecules between normal adults and those with metabolic syndrome. This DBP will drive TR&D 1 to develop new lipid and protein separation protocols and will exploit the liquid sample interface in TR&D 3 for radiocarbon measurements of HPLC and discreet liquid samples for bomb pulse dating in a BSL2 lab.
Specific Aim 1: Determine the turnover of major lipids classes using the bomb pulse. Determine the turnover of the major lipid classes (phospholipids, cholesterol, cholesterol esters, free fatty acids, monoglycerides, diglycerides, and triglycerides) in normal adults and those with metabolic syndrome. Determine if people with metabolic syndrome retain lipids longer and gradually accumulate lipids over time.
Specific Aim 2: Determine the turnover of proteins associated with lipid droplet storage and regulation.Determine the turnover of the major structural lipid droplet proteins (perilipin, adipophilin, and TIP47) and compare to the turnover of the lipids found in lipid droplets or the cells in which they reside. These proteins isolate the lipids from the cytoplasm and are associated with lipid regulation.
Specific Aim 3: Determine the turnover of structural collagen in the extracellular matrix of adipose tissue. Determine the turnover of collagen, a major component of extracellular matrix (ECM) of adipose tissue. Compare the turnover of structural collagen in adipose tissue to adipocytes.
This project seeks to identify the turnover of major components of adipose tissue to determine differences between normal and dysfunction associated with metabolic syndrome.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: DBP1 accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

We anticipate work to begin on Specific Aim 1 (Determine the turnover of major lipids classes using the bomb pulse) during the next reporting period. A HPLC method will need to be adapted for the liquid sample interface from the lipidomics literature. We also anticipate work on Specific Aim 3 (Determine the turnover of structural collagen in the extracellular matrix of adipose tissue) to occur. Published methods for harvesting collagen, a major component of extracellular matrix (ECM) of adipose tissue, will need to be modified to produce pure samples for AMS dating analysis

B.2 (DBP1 accomplishments.pdf)

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

The reduced budget and extended personal leave by DBP1 PI Kirsty Spalding has resulted in this project being put temporarily on hold.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? Not Applicable
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? NOTHING TO REPORT
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

DBP1 PI Kirsty Spalding is returning to work in March 2016 after an extended personal leave. Work on the project had not started in her absence, but we anticipate it commencing in the next couple months.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B DRAFT

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									**Total Senior/Key Person**	

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: DBP2 Absolute Quantitation of Protein Post-translational Modifications
Component Project Lead Information: Burlingame, Alma

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

This project aims to combine AMS and standard mass spectrometry technologies to quantify medically-important protein post-translational modifications in model systems. This project is unique in that it involves the collaboration of the P41-funded LLNL Bio-AMS Research Resource with the P41- funded National Bio-Organic, Biomedical Mass Spectrometry Resource Center at UCSF. This project involves both basic scientific research and technology development, and is closely linked to TR&D cores 1 and 3.
Post-translational modifications of histone proteins are key determinants of gene expression (Selvi and Kundu, 2009). Multicellular organisms consist of various cell types with identical genetic information but a high degree of differentiation, characterized by a unique pattern of gene expression for each cell type. Establishment and maintenance of these diverse expression patterns is fundamentally important for the development, function and survival of organisms. Such diverse, yet cell-type-specific gene expression patterns are achieved by epigenetic mechanisms. Among these regulatory mechanisms, histone post-translational modifications have been implicated as particularly important for defining the epigenetic status of a cell (Cortez and Jones, 2008; Gargiulo and Minucci, 2009; Selvi and Kundu, 2009).
The ability to quantify post-translational modifications at specific sites in important regulatory proteins such as histones under different biological conditions would provide important insight into how these modifications regulate biological function (Hanke et al., 2008; Kirkpatrick et al., 2005; Zee et al., 2011). Despite advances in instrumentation made in recent years, absolute quantitation of proteins and protein post-translational modifications in biological systems remains an elusive goal. Relative quantitation of post-translational modifications can be routinely accomplished with the use of stable isotope labeling procedures and mass spectrometric detection of protein modifications (Evertts et al., 2013; Gant-Branum et al., 2009; Munday et al., 2012; Ong, 2012; Wasinger et al., 2013; Xu et al., 2012). Although relative quantitation of histone modifications provides enlightening results, it is challenging to quantitate peptides with overlapping sequences and determine the occupancy for each modification site. Accelerator mass spectrometry (AMS) offers superior sensitivity and a unique ability for absolute quantitation, and therefore will be ideal for quantitative analysis of specific histone post-translational modifications (often referred to as “marks”). The unique ability of AMS for absolute quantitation will provide the enabling technology to study the occupancy of various histone modifications, and their dynamic changes under various biological conditions.
Specific Aim 1: Using 14C-AMS, measure absolute abundance of steady-state histone H4 acetylation marks in model cell types. Labeling protocols will be developed in TR&D Core 1 to provide histone H4 14C- acetylation marks measureable by liquid sample AMS. The total histone modification profile of histone H4 will be characterized in intact histones, and site occupancy will be measured on each acetylation site in peptides generated by proteolytic digestion of histone H4.
Specific Aim 2: Couple 14C-AMS and 13C-stable isotope labeling of histone H4 acetylation marks to measure absolute abundance of combinatorial modifications in model cell types. Protocols will be developed in TR&D core 1 to allow simultaneous labeling of histone acetylation marks in separate cultures with 14C and 13C. 14C-acetylation will allow absolute quantitation of selected acetylation marks, and these values can then be used to relate relative quantitation generated by 13C-acetylation to absolute quantities. This approach will enable direct or indirect quantitation and structural characterization of all histone H4 acetylation marks.
Specific Aim 3: Using 14C-AMS, measure kinetics of histone H4 acetylation in model cell types under different cell culture conditions. Rates of new histone synthesis and total histone acetylation will be measured in the presence or absence of histone deacetylase inhibitors, and in cells under different nutritional conditions. Site occupancy and acetylation rates will be measured in histone H4 peptides.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: DBP2 accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

No experiments are planned for the next reporting period.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

No significant progress was made on this DBP during the current performance period. Progress on this DBP is contingent on method developments in TR&D Core 1, which are ongoing.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?
Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?
Not Applicable
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?
NOTHING TO REPORT
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

Efforts have been focused on TR&D Core 1 method development rather than on applications proposed in this DBP.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B DRAFT

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									**Total Senior/Key Person**	

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: DBP3 Identifying Receptors of Inflammation Associated with Soluble Expoxide Hydrolase (sEH)

Component Project Lead Information:

Hammock, Bruce

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

This DBP seeks to identify the cellular receptors of the epoxyeicosatrienoic acids (EETs), the major signaling molecules in cytochrome P450 (CYP450) branch of the arachodonic acid (AA) cascade. Identification of the receptor(s) for the EETs is critical for a detailed understanding of their fundamental biology, as well as devising new pharmaceutical strategies to treat inflammatory and pain disorders. The AMS-based technology developed here will be used to identify EET receptors and is generally applicable to finding other new ligand-receptor pairs. A high affinity-low abundance receptor and lipophilic non-specific binding requires a very specific label. A radioisotope label is the best choice for specificity and owing to low abundance 14C-AMS is the best option. TR&D 1 will develop methods for receptor identification based on binding of 14C-labeled ligands to enable this DBP. Conversely, this DBP will drive TR&D 1 to develop new labeling strategies and peptide separation protocols and will exploit the liquid sample interface in TR&D 3 for high-throughput, rapid screening.
Identifying the first receptor of EETs will require a stepwise approach of constructing 14C-labeled probes and employing AMS to locate EETs followed by quantitation of binding. We propose the following series of specific aims to identify and verify the EET receptor.
Specific Aim 1: Identify cell lines and tissues that have the greatest affinity for EET. Synthesize 14C-EET from commercially available 14C-arachindonic acid (AA) for use as a probe to screen cell lines and tissues for plentiful EET receptors and low non-specific binding. Screen a variety of cell lines (endothelial, cardiomyocyte, monocyte, macrophage) and tissues (aorta, arteries, trachea) with known biological importance. Initial screening will be done by liquid scintillation counting and selected tissues or cell lines will be further screened by AMS.
Specific Aim 2: Synthesize an 11,12-EET mimic with a 14C-photoaffinity label (PAL) for EET receptor identification. Design and synthesize 14C-PAL for EET receptors identification. Test the ability of the synthesized PAL to displace 14C-EETs bound to the identified cell lines or tissues from above. In parallel to the studies below, prepare and test similar labels for ω-3-fatty acid epoxide for receptor identification.
Specific Aim 3: Use the 11,12-EET-PAL on sEH as a model system and determine orientation of substrate binding. Optimize the conditions for photoaffinity labeling of the sEH and characterize the adduct(s) using high resolution mass spectrometry (HRMS) and LC/MS-MS peptide sequencing. Test the utility of the 14C-11,12-EET-PAL to quantify sEH in individual cell types with AMS.
Specific Aim 4: Use the 14C-11,12-EET-PAL to identify potential receptor(s) for 11,12-EET in inflammatory tissues. Develop a general methodology to identify potential 11,12-EET receptor(s) in vitro or ex-vivo using AMS. Once identified, clone and express the protein(s).
Specific Aim 5: Test the hypothesis that the identified protein(s) are involved in the action of EETs as anti-inflammatory signaling molecules. Measure the binding affinity of different EET regioisomers and different chemical mediators from the AA cascade on the identified protein(s). Employ a cell-based assay using NF-κB reporter system to determine the EC50 and the mode of action of different EET regioisomers and other epoxy fatty acids on identified protein(s).
The method we propose here will be a de novo way to identify receptors for similar signaling molecules or molecular targets using AMS and 14C-PAL.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: DBP3 accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: DBP3 section B4.pdf

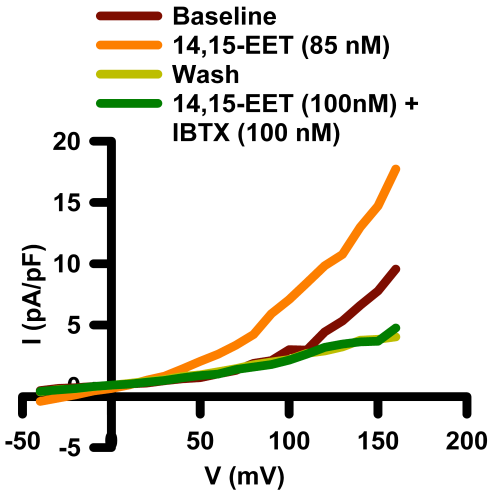
B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Dr. Sing Lee has presented the work at the following venues:
Kin Sing Stephen Lee,, Jun Yang, Lionel Cheruzel, Bruce Hammock, Ashly Lewis. Chemoenzymalc synthesis of epoxy-docosapentaeoic acids. Pacifichem 2015. Honolulu, HI. December 15-20, 2015. Poster.
Kin Sing Stephen Lee. Investigating the Mechanism on how Drug-Target Residence Time (tR) Affects in vivo Efficacy of Drug with Soluble Epoxide Hydrolase. Dept. of Pharmacology and Toxicology, Michigan State University. February 15, 2016. Seminar.
Kin Sing Stephen Lee. Investigating the Mechanism on how Drug-Target Residence Time (tR) Affects in vivo Efficacy of Drug with Soluble Epoxide Hydrolase. Biology and Biotechnology Division, Lawrence Livermore National Laboratory. March 24, 2016. Seminar.

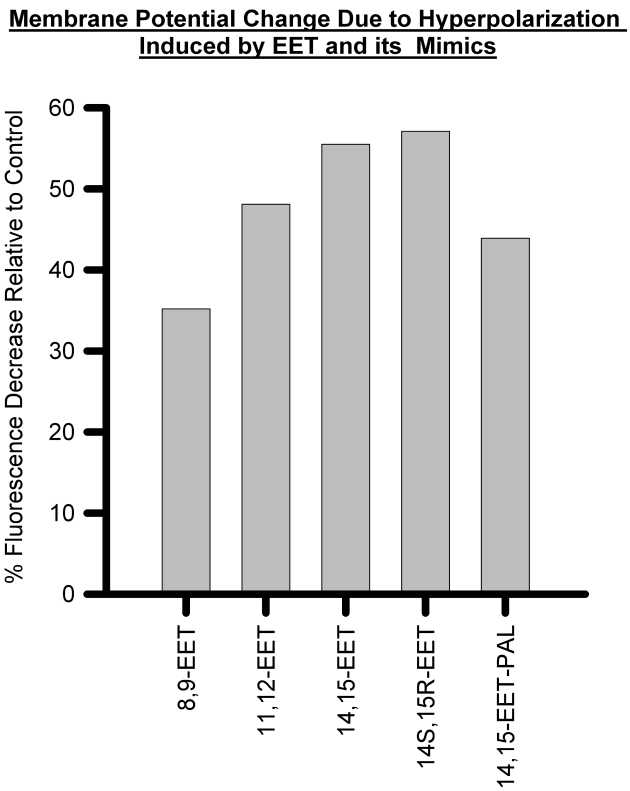
B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

We intend to synthesize the the 14C-labeled EET-PAL after confirming the potency of the 14,15 EET-PAL is comparable to 14,15 EET. The EC50 of the EET-PAL should be within a factor of 4 to 5 of the EC50 of EET in BK channel activation. Also, we will screen different cell lines for responses to EET.

Specific Aim 1: Identify cell lines and tissues that have the greatest affinity for EET.



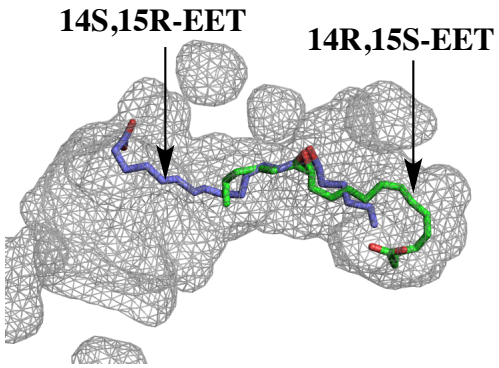
In year 1, we identified Primary Human Coronary Artery Smooth Muscle Cell (CASMC) as the model cell line to determine the potency for EET and EET mimic to activate BK_{ca} channel. We, therefore, conducted experiments to characterize if the hyperpolarization initiated by EET is due to BK_{ca} channel activation. In year 2, the EC50 of different EET-PALs mimics was measured (EC50 of 14,15-EET: 5.1 nM) and the best EET mimics will be selected as a probe for EET receptor identification. In the figure, 14,15-EET specifically activates BK_{ca} channel (IBTX is BK channel blocker) in human primary CASMC. Because we moved the system from non-primate species to human cell line, all the EET regioisomers will be re-tested. Because using patch clamp technique to determine efficacy for different EET isomers and EET-PAL mimics is very labor intensive and very time-consuming. We developed a fluorescent assay to screen different EET isomers and EET mimics using a membrane potential sensitive dye as shown in the figure. From this screening, we found that 8,9-EET is relatively less potent at 10 nM, so 8,9-EET and its mimic were not selected for further experiments. From both patch clamp and fluorescent experiment (see figures), we found that the EET and EET mimic is very potent that activates BK_{ca} channel as low as 0.5 nM.



Specific Aim 3: Use the 11,12-EET-PAL on sEH as a model system and determine orientation of substrate binding.

Aim 3 is 60% finished. we have synthesized all 3 EET-PAL regioisomer and the condition for photolabeling is optimized. The best yield of photolabeling required as fast as 1 min of irradiation at 360 nm with 10W UV lamp and this significantly minimizes the damage to the cell and to the target protein caused by UV irradiation. In collaboration with Prof. Mike Gilson at UCSD, we have docked different EET regioisomer in the binding

pocket of sEH based on the result obtained from proteomic analyses on photolabeled sEH. We found that the binding orientation of different EET regioisomers is dictated by the stereochemistry at the epoxide but is not generally believed that the specific interactions between the carboxylate group in different EET regioisomers with the binding pocket of sEH. We are likely to focus on 14,15-EET because we have a way to synthesize the specific stereoisomer at the epoxide position which could potentially enhance the selectivity for identifying the receptor.



B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

Dr. Kin Sing Lee, currently an Asst. Project Scientist in Prof. Bruce Hammock's Lab, is focusing on this project and worked with RR staff member Bruce Buchholz to develop the project into a K99/R00 application. Dr. Lee is in the second year of his K99 and Dr. Buchholz is part of his mentoring team with Prof. Hammock. Dr. Lee is currently interviewing for faculty positions.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

The project is making progress on a new approach to identifying protein receptors. It is not yet ready for technology transfer as the project is not complete.

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B DRAFT

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									**Total Senior/Key Person**	

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: DBP4 Development of Synthetic Therapeutic Proteins
Component Project Lead Information: Baker, David

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

This project aims to establish a model pipeline for synthetic protein design, development, and characterization that includes full integration of computational design of proteins with experimental testing and validation methods that are broadly applicable to the entire class of biopharmaceuticals. Specifically, this project seeks to characterize bioavailability, pharmacokinetics and pharmacodynamics of new classes of synthetic protein reagents for therapeutic applications. This project requires the sensitivity of the AMS-liquid sample interface to quantitatively trace the fate of experimental proteins and their decomposition products in rodents, and in subsequent oral microdosing studies in human subjects.
The development of protein-based therapeutics has revolutionized modern medicine (Leader et al., 2008). The exquisite specificity and selectivity of proteins distinguishes them from standard small molecule drugs. However, despite these advantages, protein therapeutics tend to be very expensive to manufacture and typically have to be administered via invasive and lengthy procedures (Leader et al., 2008). Recent advances in computational protein modeling have enabled us to design synthetic proteins to interact with cell surface target proteins in a manner similar to other protein-based therapeutics (Azoitei et al., 2012; Azoitei et al., 2011; Fleishman et al., 2011a; Fleishman et al., 2011b; Gront et al., 2011). A significant advantage of this breakthrough approach is that, while these designed proteins demonstrate the same beneficial specificity of protein therapeutics, their synthetic nature allows incorporation of additional features into their design which may improve both their manufacturability and their potential to be administered by non-invasive routes. However, there are two significant hurdles that must also be addressed before they can be used as therapeutics: 1) standard analytical methods limit our ability to gain a thorough understanding of the pharmacokinetics of the designed proteins and 2) potential immunogenicity of the designed proteins could prevent their clinical use. These hurdles are partially intertwined, since protein immunogenicity leading to generation of anti-drug antibodies can significantly alter the pharmacokinetics and pharmacodynamics of protein drugs, resulting in loss of drug efficacy (Baumann, 2006, 2009; Buttel et al., 2011; Chirmule et al., 2012; Perez Ruixo et al., 2013). The goal of this project is to to characterize bioavailability, biodistribution, pharmacokinetics, and pharmacodynamics of selected 14C-labeled synthetic proteins in rodents using accelerator mass spectrometry (AMS). This work will complement work performed by other collaborators of the Baker laboratory to characterize efficacy and immunogenicity of synthetic proteins. This proposal aims to establish a model pipeline for synthetic protein design, development, and characterization that includes full integration of computational design of proteins with experimental testing and validation methods that are broadly applicable to the entire class of biopharmaceuticals. Data obtained from the proposed experiments will help overcome one major hurdle preventing use of synthetic therapeutic proteins by providing accurate information about the quantitative biological fate of these compounds in rodents. This information can then be used to guide the design of human studies for development of these synthetic proteins for clinical use. The end goal of this project in this funding cycle is to begin oral microdosing studies in humans. Additional human microdosing studies are anticipated in future funding cycles.
Specific Aim 1: Characterize biodistribution and absolute bioavailability of orally-administered synthetic therapeutic proteins in rodents. As an initial test case, a synthetic protease (KumaMax) designed to treat celiac disease by cleaving gluten and thereby preventing an inflammatory response (Gordon et al., 2012) will be used for bioavailability and biodistribution studies in rats. Appropriate protein labeling procedures will be developed in TR&D Core 1, and animals will be administered relevant oral doses of 14C-labeled synthetic protease KumaMax. The biological fates of the therapeutic protein will be characterized and quantified in the presence or absence of gluten. This protein will be used to develop a model approach to measuring bioavailability and biodistribution of orally-administered therapeutic proteins. Additional proteins will also be designed and tested for administration by the oral route.
Specific Aim 2: Characterize pharmacokinetics, biodistribution, and pharmacodynamics of parenterally-administered synthetic therapeutic proteins in rodents. Proteins will be computationally designed for selected activities with potential therapeutic applications. Protocols will be developed in TR&D Core 1 for 14C-labeling of synthetic proteins for administration to animals. Pharmacokinetics, pharmacodynamics, and biodistribution of 14C-labeled synthetic proteins will be characterized in rodents using appropriate doses and routes of administration, including intravenous and subcutaneous. Pharmacokinetic data will then be used to determine whether therapeutic protein candidates possess suitable characteristics for further development and potential use in human clinical trials. Results from animal experiments will also be used to further refine the design of synthetic therapeutic proteins in an iterative process, with the goal of developing protein drug candidates suitable for use in human studies.
Specific Aim 3: Perform human oral microdosing studies with selected synthetic therapeutic proteins.
Proteins possessing good biodistribution properties in rodents will be labeled with 14C and microdoses will be administered to humans to determine their potential for clinical use. It is anticipated that the gluten-hydrolyzing protease KumaMax will be the first synthetic protein tested in human microdosing experiments.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: DBP4 accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

No experiments are planned for the next reporting period.

B.2 (DBP4 accomplishments.pdf)

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

No significant progress was made on this DBP during the current performance period. No samples have been provided to TR&D Core 1 for the development of separation techniques.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? Not Applicable
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? NOTHING TO REPORT
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

Baker Lab lost interest in project.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B DRAFT

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									**Total Senior/Key Person**	

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: DBP5 The Pharmacokinetics of PAHs in Humans at Environmental Levels
Component Project Lead Information: Williams, David

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

This project seeks to measure the pharmacokinetics of PAHs and their metabolites in humans exposed at environmental levels. This project requires the exquisite sensitivity of the recently developed liquid sample measurement capability for AMS along with high efficiency extraction and HPLC methods compatible with this interface.
PAHs (polycyclic aromatic hydrocarbons) are widespread pollutants that result from the burning of material such as cigarettes, diesel, coal, or gasoline. They are also found in foods such as grilled or smoked meats and cheese, or in liquor aged in charred wood barrels. In animals, high levels of PAHs can cause cancer of the skin, breast and lung. In humans, PAHs are linked to cancer and other health problems such as asthma. It is unknown how fast PAHs are absorbed into the body when eaten, or how long they stay in the body. Even though PAHs are ubiquitous environmental pollutants and are of potential concern to human health, there is little or no information on the pharmacokinetics of PAHs in humans. With the utilization of Accelerator Mass Spectrometry (AMS), it is possible to measure 14C-labeled PAHs and their metabolites in human tissues following administration of environmentally relevant doses with the results used to model their pharmacokinetics in humans. This work has the potential benefit for regulatory agencies, for as of now, all they can rely on is high-dose animal data. As PAHs are increasing in the environment, it is crucial that we have the best information possible to make informed decisions concerning exposure consequences of PAHs.
We will employ AMS to assess, for the first time ever, the pharmacokinetics of benzo[a]pyrene (BaP) in humans. We will assess the predictive power of the Relative Potency Factor (RPF) approach for risk assessment by dosing human volunteers with 14C-BaP and with a food containing a complex PAH mixture. We will evaluate the impact of genetic polymorphisms on pharmacokinetics. We expect that the pharmacokinetics and metabolic profiles will not change when we co-administer 14C-BaP and smoked salmon containing a PAH mixture. Rejection of this hypothesis would call into question a key requirement for the RPF approach for PAHs: additivity at environmentally relevant doses. Additionally, we will conduct an exploratory gene- environment interaction study with individuals exhibiting distinct pharmacokinetic parameters, metabolic profiles, and/or formation of DNA adducts. Finally, we will establish the impact of dietary indole-3-carbinol (I3C) from brussels sprouts, in humans, on the pharmacokinetics of a model PAH, phenanthrene, often used as a biomarker of environmental PAH exposure.
Pharmacokinetic data for a carcinogenic PAH in humans at environmentally relevant levels of exposure will fill an important gap in the knowledge base available to researchers and regulators. Furthermore, we expect that a test of a key requirement of the RPF approach to risk assessment by determining deviation from additivity in PAH mixtures will have a large impact in the field of PAH risk assessment.
Specific Aim 1: Conduct the first pharmacokinetic study of BaP in humans. We will dose human volunteers orally with 14C-BaP. We will ship coded blood and urine samples to Lawrence Livermore National Laboratories (LLNL), where 14C-BaP will be measured by AMS. Furthermore, we will utilize a new technology for LS-AMS (Liquid Sample AMS) to identify and quantify the major metabolites of 14C-BaP in blood and urine. This new technology will greatly increase our sensitivity and sample throughput. This work will significantly increase our ability to model and interpret BaP pharmacokinetics in humans at low exposures.
Specific Aim 2: Conduct the first evaluation of the Relative Potency Factor approach to risk assessment for PAHs in human studies. To a subset of the same volunteers, we will co-administer the same dose of 14C-BaP with smoked salmon containing a known PAH composition. The portion of fish will have an RPF equivalent to 10-x BaP alone. If there is no deviation from additivity (a prerequisite for the RPF approach), the pharmacokinetics, metabolism and 14C-BaP-DNA adducts in lymphocytes (if present) should not change.
Specific Aim 3: Conduct the first assessment of gene-environment interactions in human at environmentally relevant levels of exposure to PAHs. In this exploratory study, we will determine whether individuals exhibiting significant differences in pharmacokinetics, metabolism or DNA adduction express select allelic variants with respect to enzymes known to be involved in BaP metabolism. Results from our lab and others suggest that cytochrome (CYP)1B1 plays the predominant role in many target organs for BaP bioactivation. One genetic polymorphism impacting CYP1B1 expression and activity is the CYP1B1*3 allelic variant, present in up to 40-50% of the general population (varies with ethnicity). To our knowledge, this is the first study of its kind, assessing gene-environment interactions in risk assessment at environmentally relevant levels of exposure.
Specific Aim 4: Conduct the first assessment on the impact of phytochemicals on the alteration of the pharmacokinetics of PAHs in human volunteers. Indole-3-carbinol (I3C) is found in cruciferous vegetables such as broccoli, cabbage, cauliflower, brussels sprouts, collard greens and kale. Furthermore, it is also a popular diet supplement with antioxidant and proposed anticancer properties. We will dose human volunteers with 14C-phenanthrene with and without pre-treatments with I3C or Brussels sprouts for one week and collect plasma and urine samples at specified timepoints. We hypothesize that prior and concurrent dosing with I3C and/or Brussels sprouts will significantly alter the pharmacokinetics of 14C-phenanthrene, a PAH commonly found at high concentrations in environmental PAH mixtures and used as a biomarker of exposure in humans.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: DBP5 Acomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: DBP5 Training.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Madeen EP
New Technologies Track Human Metabolism of Polycyclic Aromatic Hydrocarbons, an Important Class of Environmental Pollutants
The Linus Pauling Institute Research Newsletter, Corvallis OR, Spring/Summer 2015, P10-11

Audience: Health promotion community, collaborators, non-academic persons interested in disease prevention/healthy aging, research benefactors, etc.

http://lpi.oregonstate.edu/research-newsletter

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Volunteer recruitment will continue though 2017. Currently, 14C-BaP samples are being analyzed by SS-AMS for metabolite formation and intervention with a characterized PAH mixture to assess the accuracy of the EPA additivity approach to PAH toxicity.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

DBP5 Acomplishments:

1. Plasma from human volunteers administered ^{14}C-BaP was analyzed on the solid sample AMS format, providing data that the inter-individual variance during replicate dosing is minimal. This data provided evidence that replicate dosing was not necessary for most volunteers, reducing the discomfort and potential risk requested of human subjects.
2. OSU Superfund Research Program (SRP) objectives: 1. Determine the pharmacokinetics of PAH BaP by AMS-moving wire interface. 2. Compare the pharmacokinetics of ^{14}C-BaP alone vs ^{14}C-BaP with the addition of a characterized PAH mixture; ^{14}C-BaP PK/PD remaining the same or becoming lesser with the addition of the PAH mixture indicates a lack of support for the PAH additivity of risk approach.
3. DNA from volunteers administered 14C-BaP was analyzed for associated ^{14}C-BaP, representing DNA adducts, providing no evidence that microdosing humans with ^{14}C-BaP led to an increase of DNA adducts at the 72 hour time point, supporting our rational of administering a *de minimus* dose.

DBP5 Training & Professional Development

Oregon State University SRP trainee Erin Madeen has visited CAMS on several occasions to prep, process, and analyze DBC plasma and urine samples under the mentorship of Resource staff. While analyzing data generated by LS-AMS, Resource Investigator, Dr. Ognibene, developed a user driven pipeline for LS-AMS data that met Erin's needs for quality and uniformity in PK analysis. She shared the dibenzo[def,p]chrysene (DBC) and BaP metabolite data with Rick Corley and Jordan Smith of Pacific Northwest National Laboratory, where she learned basic pharmacokinetic analysis. The skills that she gained during this experience contributed to the successful defense of her Ph.D. dissertation and her recruitment for a post doctoral position in drug metabolism.

Erin Madeen presented a poster at the Gordon Research Conference on Drug Metabolism in Holderness, NH on July 17, 2015 entitled, "Environmentally Relevant Microdosing of Human Volunteers For PAH Pharmacokinetics."

Oregon State University research faculty, Sharon Krueger, became proficient in FDA IND applications and human subjects clinical coordination through her affiliation with the Williams lab. She completed ethics and confidentiality training in addition to bridging relationships and collaboration with the IRB and FDA systems.

OSU undergraduate research assistant and dietetics student, Jessica Hummel, applied her training to analysis of dietary records kept by human subjects to assess compliance with dietary restrictions and to estimate the dietary intake of BaP by subjects enrolled in this project.

A poster was presented at OSU Environmental & Molecular Toxicology Research Day on January 21, 2016 entitled, "Estimated Dietary Exposure to Benzo[a]pyrene (BaP) During Clinical Dietary Restriction," by Jessica M. Hummel, Erin P. Madeen, Lisbeth K. Siddens, Sandra Uesugi, Sharon K. Krueger, and David E. Williams.

OSU undergraduate research assistants Alex Van Scoyk, Hannah You, David Sampson, and Jessica Hummel, received blood borne pathogens training and training in sample preparation through their involvement in SRP Project 1.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Data generated has led to the development and refinement of the LS-AMS analysis software developed in TR&D3.

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

The dataset generated by this project may be used by regulatory agencies to refine risk assessment for PAHs based on actual human data following exposures at environmentally relevant concentrations.

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

Yes

Exemption number(s) E4

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE Not Applicable
G.11 PROGRAM INCOME Not Applicable
G.12 F&A COSTS Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B DRAFT

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: DBP6 Microdose-based Prediction of Chemotherapy Efficacy

Component Project Lead Information:

Henderson, Paul

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

This project seeks to measure the binding of cancer drugs to their biological target, DNA, as a tool to better understand, predict and overcome drug resistance. Since these studies are aimed at minimally invasive human sampling, small sample size, compatibility with HPLC-AMS and liquid sample AMS are critical to success.
Platinum-based chemotherapy is the primary therapeutic intervention for over 300,000 cancer patients per year in the US. Although some patients are cured, as many as 50-75% of lung, bladder and breast cancer patients do not benefit from this treatment (Comella et al., 2007; Schiller et al., 2002; Silver et al., 2010; Soloway et al., 2002; von der Maase et al., 2005). There are no clinically accepted tests that predict resistance to this class of drugs. We are developing the PlatinDx assay in order to identify chemoresistance in cancer patients before they receive platinum-based therapies including carboplatin, cisplatin and oxaliplatin (Cimino et al., 2013). The assay utilizes subtherapeutic "microdoses" (~1/100th the therapeutic dose) of 14C- labeled carboplatin or oxaliplatin whose in vivo fate is measured by accelerator mass spectrometry (AMS)(Hah et al., 2006; Hah et al., 2007; Henderson et al., 2011; Wang et al., 2010). Drug-DNA damage is measured by AMS in white blood cells and tumor biopsy tissue as a predictor of therapeutic response. The hypothesis is that killing of cancer cells by platinum-based drugs requires a threshold level of drug-DNA damage, and that microdose-induced drug-DNA adducts are predictive biomarkers of whether cells can achieve that threshold during full dose therapy (Figure 1).
We propose to (1) further validate and improve the platinum-based microdose diagnostics methodologies for the ongoing clinical program (2) use the Nod scid gamma patient-derived tumor xenograft (NSG PDX) mouse model system to study drug resistance and personalized bladder cancer therapy for carboplatin and gemcitabine combination therapy (both are DNA modifying cancer drugs). This effort will reduce the sample size requirements, increase the speed of analysis and expand the research to include additional drugs and combination therapies using the world-renowned expertise of UCDCCC in human cancer studies and the evolving unique AMS capabilities at LLNL. If successful, the results will advance our understanding of drug resistance, improve cytotoxic chemotherapy, and aid the development of new companion diagnostics to enable personalized therapy according to the following aims:
Specific Aim 1: Conduct clinical pilot studies to validate microdose-induced drug-DNA adducts as biomarkers of treatment efficacy for carboplatin (lung and bladder cancer) and oxaliplatin (breast cancer). This aim will require improved sensitivity and robust protocols that are compatible with clinical applications to increasingly large patient numbers at multiple sites. Furthermore, adaptation of the current graphite-based protocols to liquid sample AMS will increase throughput and may reduce the invasiveness of biopsy procedures.
Specific Aim 2. Test the feasibility of using carboplatin in combination with gemcitabine for dual label microdosing studies of drug resistance in bladder cancer using the NSG PDX mouse model. This Aim will require the separation of multiple radiocarbon labeled drug-DNA adduct species based upon their physical or chemical properties, or enzymatic digestion and the use of HPLC-AMS.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: DBP6 Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: DBP6 Training.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Paul Henderson chaired a session on biomedical AMS at the 250th National Meeting of the American Chemical Society held in Boston from August 16-20, 2015 titled “New Approaches to the Study of Chemical Toxicology in Human Health: Accelerator Mass Spectrometry”

See article in UC Davis Medicine magazine (Spring 2015):
http://www.ucdmc.ucdavis.edu/ucdavismedicine/issues/spring2015/features/envisioning.html

Paul Henderson had a meeting with Professor Young Shin of Chungnam University (South Korea) at UC Davis regarding the possibility of expanding our clinical program to Korea on December 21, 2015.

Paul Henderson was invited on tour of South Korea to visit Seoul National University, the Korea Institute of Science and Technology, Chungnam University, Biocore Incorporated and Curachem Incorporated. He gave talks at each location and engaged the leadership in discussions regarding GMP manufacture of radiocarbon labeled drugs and clinical studies. I was invited to participate in writing a position paper for the Korean FDA in order to aid in the issuance of guidance for mirodosing studies in South Korea. The tour was from August 18-22, 2015. The report will be published in 2016.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

We plan to continue performing experiments aimed at producing materials for evaluation by the moving wire system, including gemcitabine-DNA adducts, which are of relevance to clinical research that is ongoing at UC Davis.

DBP6 Accomplishments

Major activities included analyzing quality control samples of DNA containing carboplatin-DNA adducts which range from concentrations that can be detected by liquid scintillation counting (LSC) down to those that can be quantified by AMS that cover the range expected for patient samples. These standards were measured on three AMS instruments: (1) the LLNL graphite instrument, (2) the graphite instrument at Vitalea Science and (3) the gas ion source instrument at TNO. There was no statistically significant differences in any of the measurements, but the TNO instrument was able to get the data without the introduction on any carrier carbon. This is good proof-of-concept for (1) widespread use of AMS as a diagnostic tool for patients and (2) the LLNL moving wire system, since it also has a gas ionization source, but it is expected that throughput and sensitivity will be much higher on the moving wire system. Furthermore, mice containing four different human-derived tumors were given microdoses and therapeutic doses of [^{14}C]carboplatin and [^{14}C]gemcitabine, which were measured by AMS, and the results compared to tumor response to full does cisplatin- or carboplatin-gemcitabine therapy. An additional tumor model was made resistant to gemcitabine by repeated dosing. The resistant tumor had significantly fewer gemcitabine-DNA adducts compared to the drug-sensitive parent tumor. This provides additional validation that microdose-induced drug-DNA adduct levels correlate to chemotherapy resistance. A colorectal cancer clinical trial was opened to accrual at UC Davis which aims to correlate microdose-induced oxaliplatin-DNA adducts to response to subsequent oxaliplatin-based chemotherapy. A second objective is to determine whether microdosing can be used to predict the pharmacokinetics of the therapeutic dose as a potential strategy for dose optimization. The second aim is important, since approximately 30% of patients are over or underdosed, which results in compromised efficacy and toxic side effects. Two patients have been accrued to trial so far and the protocol has been safe (no microdose-induced side effects). We also started dosing human leukemia cells with radiocarbon labeled cytarabine and anthracycline, drugs used in the common “7 + 3” induction chemotherapy regiment. Since up to 30 % of leukemia patients given this therapy die from treatment-associated toxicity, it is important to predict which patients will respond—currently an unmet medical need. The protocols from previous cell culture studies were adapted to these studies. New protocols were required, since leukemia cells do not adhere to culture plates, and the drugs are different than previously used.

DBP6 Training & Development

UC Davis Postdoctoral Fellow Maike Zimmermann was awarded a travel grant to attend and present at the 2015 “Association for Mass Spectrometry: Applications to the Clinical Lab” meeting, which will be held in San Diego on March 28, 2015.

UC Davis Postdoctoral Fellow Tiffany Scharadin was trained in clinical leukemia research methods in order to pursue feasibility experiments in a leukemia cell culture model system.

UC Davis Medical School Professor Brian Jonas participated in an internal grant proposal with Mike Malfatti (TR&D2) in autumn of 2015. Although the grant was not funded, it formed a roadmap for studying chemoresistance in leukemia that is now under active investigation on clinical samples. **Notably, Dr. Jonas is an NIH K12 funded investigator, and is using his funding to pursue AMS studies of leukemia.**

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

If the moving wire system can be made into a robust system that is portable to industry it will be highly desirable as a commercial technology for drug development and diagnostics applications that will help to improve the efficiency of drug development and make oncology more effective.

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

Yes

Exemption number(s) E4

Does this project involve a clinical trial?

Yes

If yes, is this an NIH-defined Phase III Clinical Trial?

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B DRAFT

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									**Total Senior/Key Person**	

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: DBP7 Development of Quantitative Methods for Assessing Metastatic Potential of Human Primary Tumors

Component Project Lead Information:

Loots, Gabriela

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

This project seeks to develop quantitative methods for measuring the metastatic potential of primary tumors in xenograft rodent models to identify a few metastatic cancer cells in otherwise histologically 'normal' tissues below the current detection thresholds determined by quantitative PCR. The project will drive TR&D 2 to develop methodologies capable of separating and quantifying small amounts of 14C labeled DNA. The small sample DNA analysis will exploit and drive the liquid sample interface in TR&D 3 via highly labeled, extremely small DNA samples delivered in single drops.
The inability to effectively treat metastases is the main reason for the limited progress in reducing the rates of cancer morbidity and mortality. One major drawback is the lack of quantitative assays for assessing the size and tissue prevalence of tumors in newly diagnosed individuals. Current methods for quantifying tumor burden are mainly qualitative and include measuring the gross weight of the affected organ, counting tumors on the surface of the organ, or evaluating a small sample of the organ using histologic sections. These methods are crude measures of tumor burden and size distribution, and in the case of histology, they are time consuming, difficult to process an adequate sample size and non-quantitative. Animal models of metastasis have been useful in identifying genes that regulate susceptibility to the development and progression of metastasis and helped highlight potential novel targets for drug development. In particular several small animal imaging technologies including magnetic resonance imaging, high frequency ultrasound, and optical imaging have been recently applied to this task. Each of these methods may be useful for specific research projects, based on their unique combination of resolution, image acquisition time, animal throughput, and cost-effectiveness, yet none of these modalities adequately addresses the need for rapid quantification of tumors across the entire organism, as well as accessing therapeutic effectiveness in eradicating cancer in xenograft models.
This application aims to develop high precision quantitative methods for rapidly assessing the metastatic potential of primary tumors isolated from newly diagnosed patients. The system we propose would ultimately use as a xenograf cells isolated from a small biopsy, which would first be expanded subcutaneously in vivo, in NOD scid gamma (NSG) immunodeficient mice in the presence of 14C-labeled thymidine (C14TdR). As the tumor cells rapidly divide, C14TdR would incorporate into the cancer cells genome, which would further be used to track their location, in vivo. These 14C-labeled tumors would next be delivered intravenously into NSG mice and allowed to develop metastatic cancer over the course of 6 months. At the end of the experiment, all vital organs will be collected; the DNA will be isolated and examined by AMS for the presence of 14C-signal. The labeling optimization will aim to achieve sufficient signal such that a tumor derived from a single cell could be detected by AMS, in secondary tumors, in vivo, independent of histological data. This effort would complement the microdosing AMS studies aimed at improving drug therapeutic efficacy, as well as would potentially improve upon current methodologies for evaluating tumor eradication, in vivo. The following Aims will integrate the basic science required for optimizing in vitro and in vivo pre-clinical parameters necessary for transitioning to the use of clinical samples as well as will be applied to address some key questions in cancer biology:
Specific Aim 1: Optimize 14C labeling of cancer cells in vitro and in vivo to single cell resolution. Using a highly metastatic prostate cancer cell line (PC3) as a proof of principle, cancer cells will be labeled with C14TdR in tissue culture or in vivo, as subcutaneous tumors. Labeling conditions will be optimized to determine the best 14C dose and growth conditions such that AMS can detect signal from as low as a single PC3 cell. Labeled PC3 cells will be delivered into NSG mice (1) intrafemorally or (2) intravenously and tumors will be allowed to develop for up to 6 weeks. Bones and all other soft tissues will be examined histologically and by AMS to correlate tumor histopathology with 14C AMS measurements.
Specific Aim 2. Determine whether activation of genes known to promote epithelial to mesenchymal transition (EMT) promote the metastasis of otherwise benign basal cell carcinoma tumors. Using 14C- labeling of BCCs we will aim to understand the fundamental properties of BCCs that make them uniquely adverse to metastasis in order to distinguish genetic and environmental factors that influence BCC trafficking to internal organs. BCC clones that overexpress 4 genes known to promote EMT [LoxL3; LoxL4; Twist1; Snail] will be compared to parental lines to determine if EMT activation promotes BCC metastasis to distant sites, in xenograft mouse models.
Specific Aim 3: Determine whether the cancer [seed], the host [soil] or both contribute to prostate cancer metastasis. Using genetically modified PC3 lines that over-express two activators (Wnt3A; Wnt7A) and two inhibitors (Dkk1; Sost) of Wnt signaling we will determine whether metastasis of PC3 cells varies as a function of modulating Wnt signaling in the cancer cell. In parallel experiments, we will modify the 'host' environment by administering antibodies targeting these same proteins, in PC3 xenografts and xenografts of prostate cancer cells derived from human subjects to evaluate if metastasis is increased or decreased as a function of modulating Wnt signaling in the host. The information derived will help develop new in vivo strategies for assessing the contribution of individual allelic variants, and signaling pathways to metastasis.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: DBP7 Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: DBP7 Training.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Two posters were presented at the UC Davis Cancer Center Symposium, and two abstracts were submitted to the American Association for Cancer Research Meeting in April 2016.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

During the next period we will start work on Specific Aim 2: Determine whether activation of genes known to promote epithelial to mesenchymal transition (EMT) promote the metastasis of otherwise benign basal cell carcinoma tumors. Using 14C-labeling of BCCs we will aim to understand the fundamental properties of BCCs that make them uniquely adverse to metastasis in order to distinguish genetic and environmental factors that influence BCC trafficking to internal organs. BCC clones that overexpress 4 genes known to promote EMT [LoxL3; LoxL4; Twist1; Snail] will be compared to parental lines to determine if EMT activation promotes BCC metastasis to distant sites, in xenograft mouse models.

DBP7 Accomplishments

Significant Results

This project seeks to develop quantitative methods for measuring the metastatic potential of primary tumors in xenograft rodent models to identify a few metastatic cancer cells in otherwise histologically 'normal' tissues below the current detection thresholds determined by quantitative PCR. During the last funding period we have initiated experiments outlined in Specific Aim 3: We tested different types of prostate cancer cell lines for their metastatic potential. The results are outlined below:

1. We examined the distribution of metastasized cells to tissues at 14 days and 10 weeks post injection from prostate cancer cell lines of differing metastatic potential. PC3 is a highly metastatic prostate cancer cell line; LnCap is a non-metastatic prostate cancer cell line; Hprec is a normal primary human prostate cell line. PC3 cells showed elevated ^{14}C signal in several tissues both at 14 day (a) and 10 week (b) time points when compared to non-metastatic cell lines (Figure 1).

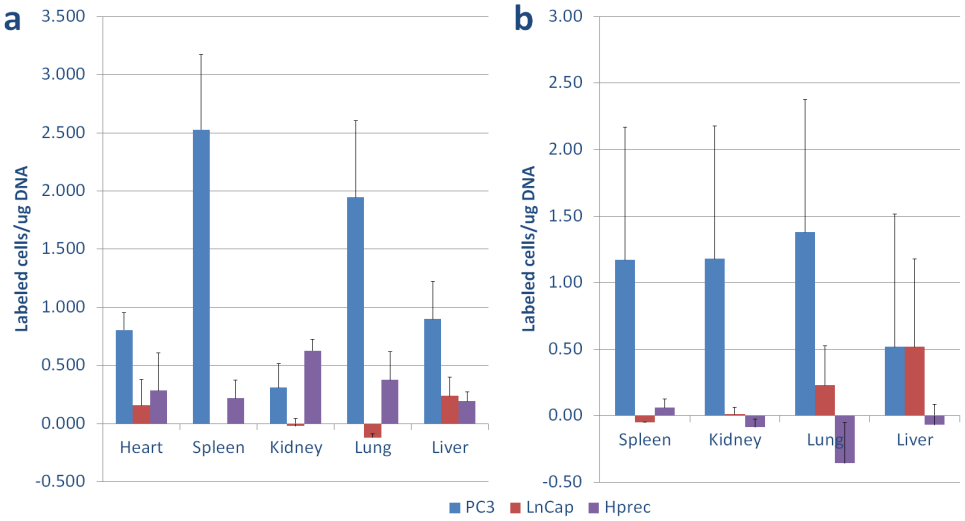


Figure 1. Determining the distribution of ^{14}C label in organs. 3 cell lines were labeled and injected into NSG mice, ^{14}C was quantified in DNA purified from organs at 14 days (a) and 10 weeks (b).

2. Stem cells have been previously hypothesized to be the 'metastatic' cells that invade distant organs to form secondary tumors. In this aim, we stained PC3 for intracellular aldehydes [a widely accepted method for identifying cancer stem cells], sorted the cells with high expression, labeled them with ^{14}C, and injected into NSG mice to determine metastatic level (Figure 2). In particular, we found a population of aneuploid stem cells that have increased size and levels of genomic content. Both diploid and aneuploid cancer stem cells were

sorted, and sublines were established. We found both these sublines to display higher incidence of metastatic tumors to the lungs.

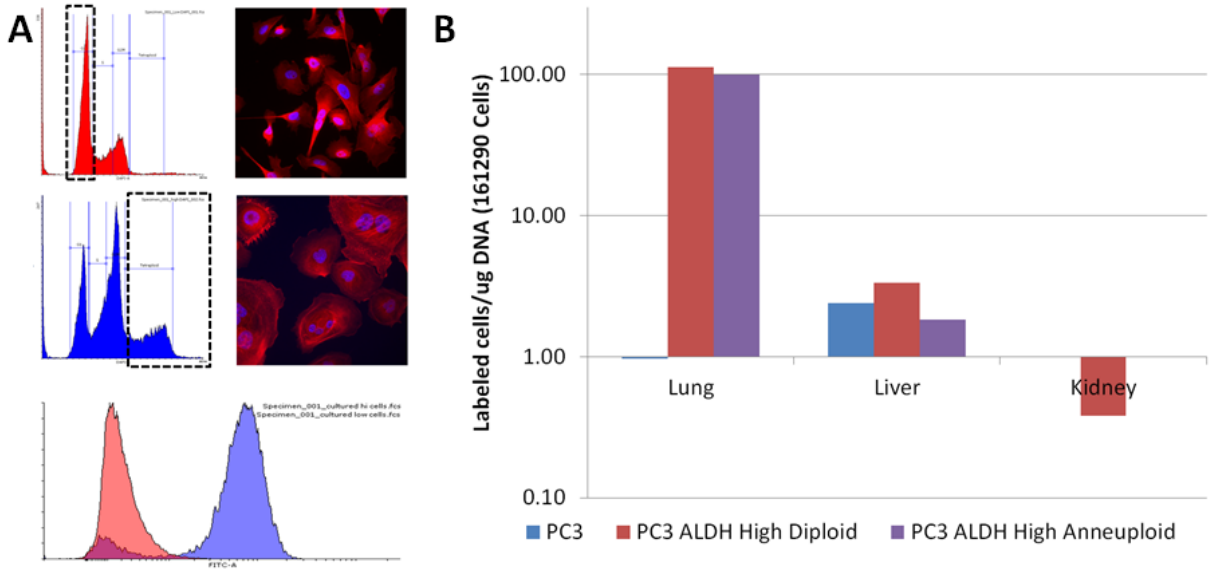


Figure 2. Cancer stem cell metastasis. PC3 stem cell sublines were generated using ALDH staining followed by cell sorting (A). Two sublines that had high ALDH retention were further separated by ploidy and analyzed for metastatic potential after 14 days incubation, *in vivo*. Cell signal is presented on a logarithmic scale. Both diploid and aneuploid PC3 cells with high ALDH retention (cancer stem cell) were found in higher concentration (~50 fold) in the lung compared to the PC3 control population.

The significant findings of our research in this funding period is that initially we can quantify metastasis based on the number of labeled cells identified in metastatic tumors. Whereas most tumors were previously estimated to stem from 1-2 cells, it is clear that cells lines that are 'more metastatic' infiltrate distant organs more readily to form larger, more aggressive tumors.

DBP7 Training & Development

Three members of our group [two graduate students and a technician] participated in the UC Davis Cancer Center Symposium where they presented two posters and had the opportunity to interact with clinical oncologists and discuss collaborative studies and extensions of the developed application of C-14 labeling in xenografts.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

We have developed a new method for quantifying metastasis. A manuscript has been drafted, and will be submitted to a peer reviewed journal.

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

File uploaded: DBP7 Resource Sharing.pdf

C.5.b Resource sharing

NOTHING TO REPORT

DBP7 Resource Sharing

Genetically modified cell lines we generate in this program will be freely available to academic researchers.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

We will explore technology transfer applications of our methodology with local biotech companies and with the UC Davis Cancer Center.

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B DRAFT

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									**Total Senior/Key Person**	

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: Collaboration and Service

Component Project Lead Information:

Buchholz, Bruce

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The Collaboration and Service (C&S) Projects are joint efforts with collaborators that use the more established Center capabilities to solve important biological questions. Currently, there are over 20 active collaborative and service projects and as many as 75 investigators affiliated with the Center. These projects not only take advantage of the Center's unique technology but also help evaluate, focus, and prioritize technology development activities.
Collaborative research projects are those that involve substantial intellectual interactions among the Resource and the collaborating scientists. Ideally, the collaborative scientist presents the Resource with a current research problem that is in the core path of a funded scientific program. The collaborator may have tried several analytical approaches to the identified problem but had not been able to obtain the sensitivity, precision, or accuracy of quantitation that is required to remove the problem from the research path. Collaborative projects are those collaborations, which, while requiring intellectual input from the Resource staff for the design, execution or analysis of data, do not drive technology development to the level of being a DBP. Collaborative projects may in the future become DBPs if it is determined that significant technology or methods development is needed. Likewise, DBPs may become collaborative projects when the technology or methods development needed is reduced to practice.
A service project is one that requires teaching, straight forward sample analysis, data analysis and data reduction. Service projects generally involve activities that the Resource Staff have done before or know how to do with little real new thought. The Service component of the Accelerator Mass Spectrometry Resource will offer the capability of analyzing biological samples for radiocarbon and/or tritium content.
We are currently highlighting seven collaborative projects with investigators distributed around the US and the world that will utilize, and benefit from, AMS for addressing important areas of biomedical research. The seven highlighted projects include diverse applications involving diverse specimens that include humans, human cell lines, and mice.
CP1: Feasibility of nanoparticle –mediated paclitaxel delivery for phase 0 clinical studies
Investigators: Kit Lam/ Paul Henderson, UC Davis
Paclitaxel (Taxol) is a standard and effective chemotherapeutic for many cancer types. Since paclitaxel (PTX) has very limited solubility in water, the formulation of this drug requires Cremophor EL, which causes significant side effects. We proposed to use a novel water-soluble nanoparticle-based formulation to enable better drug delivery of PTX. The goal of the current study is to advance the use of PTX-nanoparticles into Phase 0 clinical studies by using [14C]paclitaxel to label the nanoparticles followed by absorption, distribution, metabolism and excretion (ADME) studies.
CP2: Analysis of Cold War 14C levels in DNA from Human Prostate Tissues
Investigator: Donald Vander Griend, University of Chicago
Central to our understanding of the etiology and progression of prostatic disease is a firm knowledge of prostate tissue turnover and the formation of new cells. The recent technological advances using AMS to measure Carbon-14 levels in tissues as a result of the above-ground atomic bomb tests present a unique opportunity to address important unanswered questions in prostate research regarding growth, turnover, and disease etiology. Moreover, such C-14 detection would facilitate studies on slowly dividing and quiescent cell populations in the prostate such as stem cells.
CP3: Determining the age of cerebrovascular pathologies
3A: Determination of the age of ruptured and unruptured intracranial aneurysms Investigator: Nima Etminan, Heinrich-Heine University, Dusseldorf, Germany
The goal of this study is to establish a method to date ruptured and unruptured intracranial aneurysms in patients treated with aneurysm clipping and excision using 14C analysis of aneurysm collagen. We have established sample preparation methods that ensure that the tissue is ultra-pure and not mixed with other tissues or cells. The experiments in this collaboration are designed to test the hypothesis that unruptured aneurysms are older than ruptured aneurysms. Clinically, this suggests that patients with incidental aneurysms and cardiovascular risk factors should be monitored more closely using serial MR imaging.
3B: Determination of the age of arteriovenous malformations (AVMs) Investigator: Daniel Cooke, UCSF
The objective of the current study is to establish a method to date arteriovenous malformations (AVMs), which is now possible with the availability of AMS. Brain AVMs (BAVMs) are complexes of tortuous, tangled vessels with fistulous connections between arteries to veins without a true capillary bed. We will employ retrospective birth dating of cells to measure the approximate age of arteriovenous malformations (AVMs). By measuring the average age of DNA and collagen from AVMs we will determine molecular turnover and elucidate the progression and longevity of AVMs.
CP4: Transport of 14C-TCC Across the Placental Barrier
Investigator: Heather Enright, LLNL
Background and Significance:
Studies have demonstrated a variety of chemicals are not removed in wastewater treatment plants and are released back into the water supply and environment at low concentrations. Endocrine-disrupting compounds (EDCs), frequently found in the water supply, may not only affect the functions of the endocrine system, but also have the potential to bioaccumulate and transfer from mother to offspring in utero or during lactation. In this project we are quantifying the transfer of an EDC, triclocarban (TCC), from mother to offspring both in utero and through lactation using AMS.
CP5: Quantitative Analysis of Cell Turnover: a Strategy for Personalizing Leukaemia Treatment
Investigator: Karen Brown, University of Leicester
B-Cell chronic lymphocytic leukaemia (CLL) is a heterogeneous disease with a variable clinical course. This project aims to examine the potential clinical value of a newly-designed technique to measure CLL cell birth and death rates in vivo. In this approach, [14C]-labelled aspartic acid is incorporated into the DNA of actively proliferating CLL cells. The degree of 14C incorporation and subsequent loss of labelled cells from the circulation of an individual may identify prospectively patients destined to develop progressive disease.
CP6: Quantification of 14C by Optical Spectrometry
Investigator: Ted Ognibene, LLNL
Quantification of 14C via optical spectroscopy offers an approach that eliminates many of the shortcomings of an accelerator-based system. This technique exploits the selectivity of the ro-vibrational transitions in the infrared spectral region and their isotopic shifts. The

sensitive infrared-based approach utilizes an intra-cavity laser technique to increase the optical path-length called Cavity Ring Down Spectroscopy (CRDS). Using CRDS, we propose to develop a table-top sized spectroscopic method to quantify 14C in milligram sized biological samples at levels down to the isotopic natural abundance.
CP7: Pharmacogenomics of Drug Metabolism in Skeletal Biology
Investigator: Gabriela Loots, LLNL
While it is acknowledged that nongenetic factors such as age, health status, co-therapies and nature of the disease can all influence the outcome of drug treatment, it has been estimated that genetics account for up to 90 percent of variability in drug disposition and effects. One of the main objectives of our collaborative proposal is to couple high precision metabolic phenotyping with skeletal phenotyping in the context of bone metabolism to identify genetic mutations that contribute to different pharmacologic outcomes in osteoporosis.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: collaboration projects progress 2016.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Collab Proj B4.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

CP1:
Nothing to Report

CP2: Analysis of Cold War 14C levels in DNA from Human Prostate Tissues
Nothing to Report

CP3: Determining the Age of Cerebrovascular Pathologies
Work was presented to a clinical audience.
Nima Etminan, Unravelling the age of the physiological and pathological cerebrovasculature, Joint meeting, American and German Academy for Neurosurgery, Heidelberg, Germany, October 10, 2015.

CP4:
Work was presented at two international conferences and a departmental seminar at UC Davis.
Enright H, Falso M, Lao V, Malfatti M, Kuhn E, Hum N, Shi Y, Haack K, Kulp K, Buchholz B, Loots G, Bench G, Turteltaub KW. Perinatal exposure to triclocarban results in altered lipidMetabolism. American Chemical Society national Meeting, Boston, MA. August 2015.
Enright H, Malfatti M, Buchholz B, Ognibene T, Turteltaub K. Assessing Responses to Environmentally Relevant Doses Using Accelerator Mass Spectrometry. International Society of Exposure Science, Henderson, NV. October 2015.
Enright H. Unique LLNL technologies and their applications in toxicology research. UC Davis, Department of Biomedical Engineering Seminar Series. Davis, CA. October 29, 2015.

CP5:
Nothing to Report

CP6:
Results have been presented in a poster session and two seminars.
McCartt AD. Measurements of Carbon-14 with Cavity Ring-Down Spectroscopy. LLNL Post-doc Poster Symposium, Livermore, CA. June 19, 2015.
McCartt AD. Laser-Based Measurements of 14C. Presentation, LLNL CAMS, Livermore, CA. June 19, 2015.
McCartt AD. Laser-Based Measurements of 14C. Status Update, Picarro, Santa Clara, CA. August 11, 2015.

CP7:
Nothing to Report

Other Collaborations:
Other collaborations produced 4 peer-reviewed publications and 1 presentation at Scientific Meetings or seminars listed below.

Maze I, Wenderski W, Noh KM, Bagot RC, Tzavaras N, Purushothaman I, Elsässer SJ, Guo Y, Ionete C, Hurd YL, Tamminga CA,

Halene T, Farrelly L, Soshnev AA, Wen D, Rafii S, Birtwistle MR, Akbarian S, Buchholz BA, Blitzer RD, Nestler EJ, Yuan ZF, Garcia BA, Shen L, Molina H, Allis CD. Critical Role of Histone Turnover in Neuronal Transcription and Plasticity. Neuron. 2015 Jul 1;87(1):77-94. doi: 10.1016/j.neuron.2015.06.014. PMCID: PMC4491146

Wettersten HI, Hakimi AA, Morin D, Bianchi C, Johnstone ME, Donohoe DR, et al. Grade-Dependent Metabolic Reprogramming in Kidney Cancer Revealed by Combined Proteomics and Metabolomics Analysis. Cancer Res. 2015;75(12):2541-52. Epub 2015/05/09. doi: 10.1158/0008-5472.CAN-14-1703. PubMed PMID: 25952651; PubMed Central PMCID: PMC4470795.

Tong T, Ondov JM, Buchholz BA, VanDerveer MC. Contemporary carbon content of bis (2-ethylhexyl) phthalate in butter. Food Chem. 2016 Jan 1;190:1064-8. doi: 10.1016/j.foodchem.2015.06.053. Epub 2015 Jun 20. PMID: 26213077 [PubMed - indexed for MEDLINE]

Hooper-Bui LM, Kwok ES, Buchholz BA, Rust MK, Eastmond DA, Vogel JS. Insecticide Transfer Efficiency and Lethal Load in Argentine Ants. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:665-669. PMCID: PMC4615608

Abstracts & Presentations
Carratt SA, Buchholz BA, Ding X, Van Winkle LS. Naphthalene DNA Adduct Formation in Ex-Vivo Rodent Tissues. Society of Toxicology Meeting, New Orleans, LA. March 14, 2016.

Buchholz BA, Beckett L, Turteltaub KW, Bova GS. Cancer Metastasis Aging by Carbon 14 Dating. UC Davis Cancer Center Research Symposium, Sacramento, CA. October 2015.

Mortimer M, Petersen EJ, Buchholz BA, Orias E, Holden PA. Separation of Bacteria, Protozoa and Carbon Nanotubes by Density Gradient Centrifugation. Emerging Contaminants Summit, Westminster, CO. March 1-2, 2016.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

CP1: Feasibility of nanoparticle –mediated paclitaxel delivery for phase 0 clinical studies
Investigators: Kit Lam/ Paul Henderson, UC Davis
The results of this project are being summarized in a paper currently in draft. The data may be used for a proposal with goals to optimize the formulation, dosing and sample collection protocols in animal studies for ultimate extension to human studies.
CP2: Analysis of Cold War 14C levels in DNA from Human Prostate Tissues
Investigator: Donald Vander Griend, University of Chicago
The long term goal of this project is to elucidate the rate of tissue turnover in human prostate and seminal vesicle tissues, and to determine whether such differences in 14C content can 1) enable mechanistic studies to understand prostate tissue homeostasis and stem cell function, 2) to understand the stromal vs. epithelial origins of prostatic hyperplasia; and 3) be utilized to develop genetic clinical parameters that correlate with rates of cellular replacement. The objectives of this proposal are to determine the differences in genomic DNA 14C content between prostate zones, stroma vs. epithelia, and to optimize cell fractionation approaches to analyze specific prostatic cell types, including prostate stem cells. Our central hypothesis is that prostate tissue has a more rapid turnover than seminal vesicle tissue, and that during BPH the prostate stroma undergoes rapid cellular turnover leading to more rapid turnover of prostate epithelia.
CP3: Determining the age of cerebrovascular pathologies
3A: Determination of the age of ruptured and unruptured intracranial aneurysms Investigator: Nima Etminan, Heinrich-Heine University, Dusseldorf, Germany
Approximately 85 cerebral and internal carotid arteries were obtained and measured for 14C/C since the last paper was submitted. The entire research team is in the process of analyzing these results for the next publication now. We are also collaborating with Dr. Daniel Cooke of UCSF (collaboration 3B) to apply our elastin extraction protocol on arteriovenous malformations (AVMs) for bomb pulse dating. We anticipate continuing examination of turnover in other cerebrovascular pathologies over the next few years.
3B: Determination of the age of arteriovenous malformations (AVMs) Investigator: Daniel Cooke, UCSF
We will employ retrospective birth dating of cells to measure the approximate age of arteriovenous malformations (AVMs). AVMs are retrieved during surgery at UCSF and enter the tissue bank. Elastin and DNA will be extracted and then analyzed for 14C/C by AMS to determine the age of structural protein and DNA using the bomb pulse. By measuring the average age of DNA and elastin from AVMs we will determine molecular turnover and elucidate the progression and longevity of AVMs.
A collaborative paper combining artery and AVM dating is in draft form and will be submitted summer 2016.
CP4: Transport of 14C-TCC Across the Placental Barrier
Investigator: Heather Enright, LLNL
This relatively modest pilot study demonstrates the unique capability of AMS to quantify exposure to environmental toxins at relevant exposure levels. This is a leap forward for the field; many researchers are attempting to perform similar studies in cell culture and animal models however they are forced to use much higher concentrations of potential EDCs because they do not have a detection system with the sensitivity of AMS. Only AMS allows studies that replicate chronic exposure to very low levels of potentially biologically active contaminants in the drinking water. The success of this study will enable future work, examining a wider dose range for TCC as well as understanding the effects of other EDCs.
CP5: Quantitative Analysis of Cell Turnover: a Strategy for Personalizing Leukaemia Treatment
Investigator: Karen Brown, University of Leicester
These experiments are currently on hold while Prof Brown is on family leave through September 2016.
CP6: Quantification of 14C by Optical Spectrometry
Investigator: Ted Ognibene, LLNL
This approach will lead to a new instrument for 14C analysis that will be significantly less expensive, smaller in size and easier to use than AMS, which is the current method of choice for high sensitivity radiocarbon measurements. The proposed system would cost $300,000 and has a table-top sized footprint as compared accelerator based systems, which cost $2,000,000 and require 400 square feet of space. No specialized facilities or expertise would be needed to operate and maintain the proposed system and BS level

laboratory personnel could oversee the analysis. Importantly, these improvements would allow studies to be carried out on the metabolism, disposition, fate and mass balance of: 14C -labeled therapeutic entities for drug development; potential toxicants for risk assessment; and analysis of biomarkers for personalized medicine at much lower cost than with current accelerator based AMS approaches. Given the cost and simplicity of operation, it should also allow much greater access to high sensitivity 14C analysis than is now available; any biomedical or biochemistry laboratory could afford to operate such an instrument.
CP7: Pharmacogenomics of Drug Metabolism in Skeletal Biology
Investigator: Gabriela Loots, LLNL
In future experiments, labelled stem cells will be delivered in in animals who have undergone long-bone fractures, and we will harvest the fracture callus at various time points to determine the IV injected stem cells home to the site of bone repair, and participate in bone regeneration.

CP1: Feasibility of nanoparticle –mediated paclitaxel delivery for phase 0 clinical studies

Investigators: Kit Lam/ Paul Henderson, UC Davis

We repeated a control experiment in which rats were dosed with [^{14}C]paclitaxel, which enabled comparisions to the pharmacokinetics and biodistribution data for nanoparticle-based formulations of paclitaxel. The data set is now complete and is being prepared for publication.

CP2: Analysis of Cold War ^{14}C levels in DNA from Human Prostate Tissues

Investigator: Donald Vander Griend, University of Chicago

We submitted a R21 grant proposal to NIDDK entitled "Bomb Pulse Carbon-14 Dating of Prostate Tissues to Elucidate the Origins of BPH" in October 2015 to fund the development of a robust procedure for dating formalin fixed paraffin embedded (FFPE) specimens. The proposal was scored and forwarded to council.

CP3: Determining the age of cerebrovascular pathologies

Investigator: Nima Etminan, Heinrich-Heine University, Dusseldorf, Germany; Daniel Cooke, UCSF

While acquiring data for CP3A on collagen dating of aneurysms (Etminan et al, 2014) it became clear that normal arteries sought as controls have very low collagen content, so little that it was not possible to make viable samples for AMS analysis. Initial attempts by CP3B to isolate collagen from arteriovenous malformations (AVMs) likewise produced very low yields of collagen. A procedure to isolate pure elastin from cerebral arteries and AVMs was developed in collaboration with both CP3 groups. The elastin from approximately 85 cadaveric arteries and 15 AVMs has been dated and analysis with modeling is underway to determine structural protein turnover in arteries in health and disease states.

CP4: Transport of ^{14}C-TCC Across the Placental Barrier

Investigator: Heather Enright, LLNL

The major activities over the last year included identifying TCC and metabolites using ultra high performance liquid chromatography (UPLC) and AMS in exposed offspring, manuscript submission and disseminating this research through conferences.

:

In our previous experiments, we found in mice that TCC (100 nM) does effectively transfer from mother to offspring at environmental concentrations both transplacentally and via lactation when administered through drinking water. We investigated whether or not the signal observed for this transfer (^{14}C), was associated with TCC or its metabolites. Using UPLC and AMS, we identified that ~30% of ^{14}C was TCC, the remaining 70% was associated with metabolites for our lactation exposure group. This data was included in a revised manuscript and was submitted to Toxicological Sciences.

CP5: Quantitative Analysis of Cell Turnover: a Strategy for Personalizing Leukaemia Treatment

Investigator: Karen Brown, University of Leicester

- *U2932 cells were incubated with [^{14}C]-aspartic acid and reseeded into media containing bendamustine, a chemotherapeutic drug, to determine whether metabolic labeling of DNA can be used as a way to monitor cytotoxicity in different subpopulations. Cell staining and sorting of populations prior to AMS analysis is currently in progress.*

- *Ongoing work involves xenograft studies using human derived DOHH2 cells which have been injected into NOD/SCID mice. Mice are being closely monitored and those showing signs of disease are given a dose of [^{14}C]-aspartic acid. Animals are then sacrificed at different time points to assess the degree of [^{14}C] incorporation into cells and subsequent loss from the circulation up to 2 weeks post dosing. Blood, spleens and bone marrow are collected and processed before freezing. Samples are stained with antibodies to allow sorting of DOHH2 human cell populations by flow cytometry, followed by DNA extractions and subsequent analysis by AMS.*

- *Significant results: Results from U2932 cell incubation studies show a difference in the levels of [^{14}C]-incorporation into the DNA between the subpopulations of cells. This indicates that it may be possible to distinguish between the turnover of cell subpopulations using this method. The ongoing xenograft studies will determine whether this phenomenon also translates to cells in vivo.*

CP6: Quantification of ^{14}C by Optical Spectrometry

Investigator: Ted Ognibene, LLNL

The beginning portion of this funding period was dedicated to the difficulties of operating a Cavity Ring-Down Spectrometer (CRDS) at low temperatures (235K). This effort started in the previous funding period when most of the engineering design work was completed, and moved on to operational problems, such as temperature control, gas introduction, and a nitrogen purge for frost prevention. While each of these issues required some optimization, none of them caused significant delays. After completing these tasks, the spectrometer was capable of measuring discrete carbon dioxide gas samples at low temperatures (235K-255K) and low pressures (0-100torr). These conditions greatly reduce the interference from surrounding carbon-dioxide isotopologues, which enables the carbon-14 measurement.

At this point, efforts were focused on removing or mitigating sources of noise from the measurement, thereby increasing the sensitivity. This becomes more difficult as you approach the noise floor, and for this reason, a majority of the effort this funding period was dedicated to this task. After great effort, we characterized the system with previously-measured, ^{14}C-elevated glucose samples. Currently, the system has a limit of detection of ~0.3 modern, limit of quantification of ~0.7 modern, and a precision of ~0.25 modern at one modern. The spectrometer has a linear response from zero to 750 modern and has memory retention less than one in 5000. These specifications are adequate for biological measurements.

Most recently effort has been expended on data collection. Liver samples from a guinea-pig, pharmacokinetic data set were measured by the CRDS system, and the values accurately reproduced AMS results. This demonstrates proof-of-concept for a CRDS system capable of measuring carbon-14 for biological studies.

CP7: Pharmacogenomics of Drug Metabolism in Skeletal Biology

Investigator: Gabriela Loots, LLNL

We have been optimizing conditions for labeling actively dividing cells in vivo. We have injected IV, 3 strains of mice (C57bl6; Lrp5^{KO}; Sost^{KO}) with two concentrations of C14-thymidine [350 and 700 pCi] and harvested organs 14 days post injection, blood, bone marrow, and different regions of bones [see attached experimental design]. We detected AMS signal primarily in the vertebrae, the bone marrow, ribs, calvaria and pancreas of Sost^{KO} mice, suggesting that these tissues have significant cellular turnover, in this strain of mice.

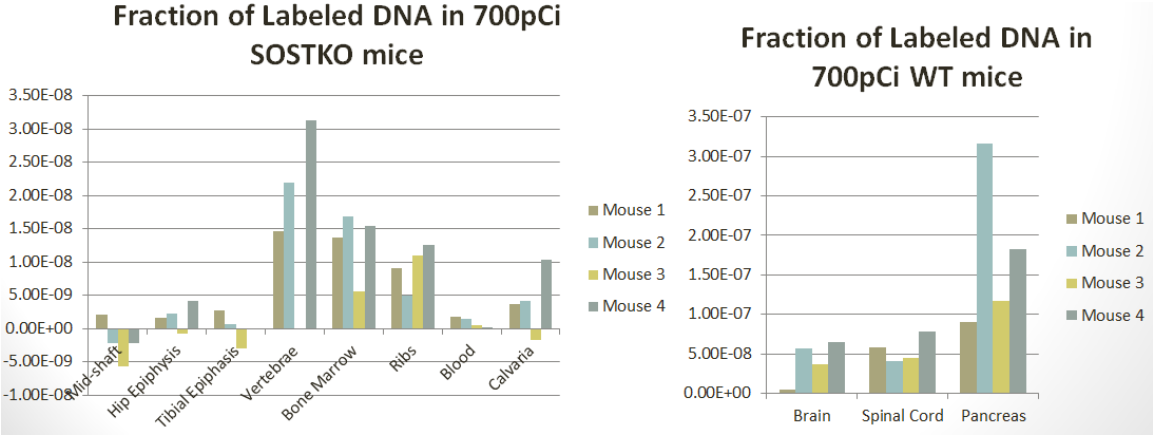


Figure 1. Sost^{KO} mice received 700pCi of C14-thymidine intravenously, and DNA was collected from tissues after 14 weeks.

In addition we used C14-thymidine to determine if Wnt3A stimulates bone formation by accelerating osteoblast proliferation. We found that Wnt3A treated calveria had significantly higher level of C14, suggesting increased bone formation is in part caused by increased proliferation.

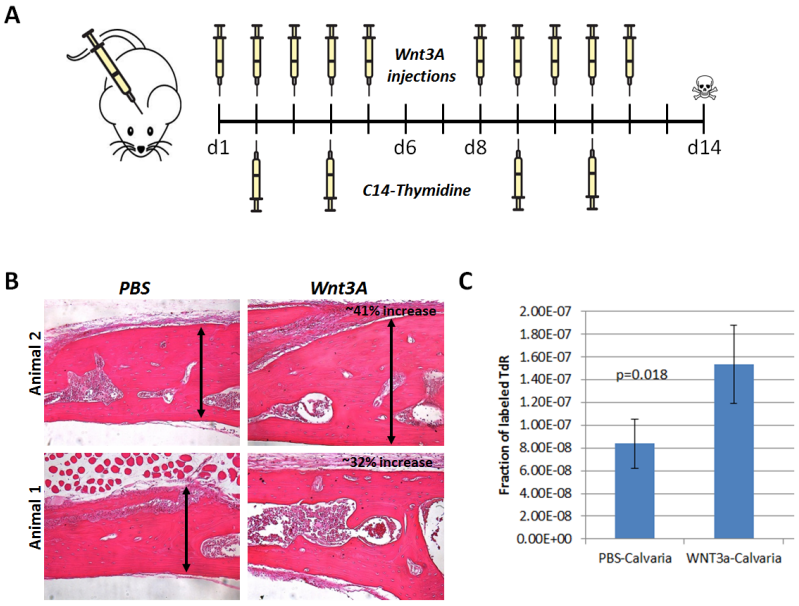


Figure 2. Bone formation calvaria assay. Mice received daily injections of wnt 3A, and twice weekly of C14-thymidine (A). At day 14, the calvaria was examined histologically (B), as well as the DNA was quantified for C14-level (C). Wnt3A calvaria had more bone mass and increased proliferation.

Section B.4

CP1:
Nothing to report

CP2:
Nothing to report

CP3:
Development of the elastin purification protocol was an iterative process between forensic medicine doctor (Dr. Christian Matzenauer) in Düsseldorf, Germany and Bruce Buchholz at LLNL.

CP4:
Heather Enright attended two conferences last year to present this work.

CP5:
Nothing to Report

CP6:
Alan McCartt began training for the purification of gaseous biological samples.

CP7:
Nothing to Report

Other collaborations:

Dr. Aboud completed a postdoctoral fellowship involving work at LLNL performing metabolic tracer experiments and sample preparation procedures for AMS analyses.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

File uploaded: resource allocation 2016.pdf

The Principal Investigator, Kenneth W. Turteltaub, makes Resource allocations with advice from the Internal Executive Committee and the External National Advisory Committee (refer to Advisory Committee pages for list of members). The Internal Executive Committee members are taken from the Resource's key personnel list. The Internal Executive Committee, along with Resource staff and associated members of the Lawrence Livermore National Laboratory (LLNL) Center for Accelerator Mass Spectrometry, meet weekly to discuss project effort and prioritization, review collaborative/service requests, and deal with other issues related to Biomedical AMS. Sample analysis requests are prioritized and scheduled at these meetings and resource staff communicate the schedules to the collaborators so that they can plan their research accordingly.

The Resource makes Biomedical AMS available to the research community through Collaborative and Service subprojects. The operational procedures for access to the Resource are available to the public on the Resource website at http://bioams.llnl.gov/. Prior to formally applying for access to the Resource, an investigator must discuss a proposal concept with an appropriate Resource staff member for evaluation of project compatibility with AMS. Only after pre-screening is an application for access to the Resource submitted to the Resource Administrator. This process has served to eliminate the need to reject applications. New subprojects, which have been accepted into the Resource, are categorized as Collaborative or Service. Collaborative subprojects align with the scientific focus of the Technology Development projects and Resource members play a key role in the project. They have the potential to become Driving Biomedical Projects, if the technology needs of the project are widely applicable to the biomedical community at large. All other projects are classified as Service. Service subprojects are supported by the Resource for initial feasibility and assisted as needed to ensure successful utilization of AMS in furthering the investigator’s studies. Individual Resource staff members provide oversight to all Collaborative and Service subprojects, as they relate to their areas of research interest and expertise.

The Resource had three new formal applications for collaboration during the reporting period. These applications, entitled “Geographic variation in human tooth size on islands: tooth wear rates in a feral goat (Capra hircus) proxy” led by Richard Madden at the University of Chicago, “Development and optimization of a blood-brain barrier permeable AChE reactivator” led by Carlos Valdez at LLNL, “The relationship between activity and post-mitotic DNA damage at a single neuron level “ led by Jerold Chun at The Scripps Research Institute, were approved as new collaborative projects.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-8631

RESEARCH & RELATED BUDGET - SECTION A & B **DRAFT**

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									**Total Senior/Key Person**	

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: Training
Component Project Lead Information: Kulp, Kristen S

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Training in the potential applications of AMS is critical to ensuring successful use of this technology by all of our collaborators. Goals for training include: teaching new investigators about how to most effectively design their experiments to obtain usable data; hands on training for high quality sample preparation, that is free from background contamination; and hands on training in the use of the spectrometer and liquid sample interface. Training is also a mechanism for informing potential collaborators about the availability of resources and equipment. We strive to provide training opportunities via multiple mechanisms, including: web-based training, which is available on our website; phone and email discussions with project investigators; in person project discussions and through postdocs and graduate students that are either directly associated with the Resource or are visitors from other labs.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: training accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: See Overall B4.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

See Dissemination component

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Effective training is critical to ensuring successful application of AMS in Biomedical Research. In the future, as the liquid sample interface becomes routine, we anticipate that more investigators will be coming onsite, participating in hands on training and running their own samples. To meet these expanded capabilities we plan to increase our onsite training through greater encouragement of investigators and students or junior members of staff to come to the Resource for 2-3 days in the initial stages of planning to discuss experimental design and see the instrument in purpose. If travel is not possible, we will make use of phone or video conferencing for these purposes. The goal for the entire grant period is to train at least 25 new investigators, including one investigator from each DBP, plus other collaborating investigators as needed.
Other training goals include:
Developing a standard AMS training workshop for on-site, or off-site, to be use at professional society meetings.
Hold workshops oriented to both generate interest in Biomedical AMS and to discuss the experiences of our users.
Update and develop new written documentation and protocols to make available to investigators, which will help collaborators to design a successful AMS experiment.

AMS TRAINING:

Training in the potential applications of AMS is critical to ensuring successful use of this technology by all of our collaborators. Our training activities are focused on: teaching new investigators about how to most effectively design their experiments to obtain usable data; hands on training for high quality sample preparation, that is free from background contamination; and hands on training in the use of the spectrometer and liquid sample interface. Training is also a mechanism for informing potential collaborators about the availability of resources and equipment and the continuing science that can be done with AMS. We strive to provide training opportunities via multiple mechanisms, including: 1) web-based training, which is available on our website; 2) phone and email discussions with project investigators; 3) in person project discussions and 4) through postdocs and graduate students that are either directly associated with the Resource or who are visitors from other labs. Activities in each of these areas will be discussed below.

1) Web- based training

The Resource's training manual and associated test continue to be a key tool, used to train investigators working in the Resource's laboratories and to verify their knowledge of the policies, practice and procedures. We have recently developed a new Web based AMS tutorial for new investigators and others interested in learning about AMS. This module provides a history of AMS and how it works, how and why it can be applied, a brief introduction to sample preparation and the liquid sample interface, guides for calculating the isotopic dose, and an example of a previous experiment. There is also a test to check the user's comprehension of the presented material. The general website can be found at https://bioams.llnl.gov/ and the training page can be found in the menu across the top of the page.

2) Phone and email discussions

We have frequent phone and email discussions with project investigators and supply them with all of the necessary written protocols for their needs. We use this mechanism to instruct on a variety of topics, including: how to set the levels of radiocarbon in a study; how to avoid contamination; and how to evaluate laboratories/equipment background isotope levels by taking surface and aerosol samples prior to initiating studies utilizing AMS.

3) In- person project discussions

In- person project discussions encourage and facilitate collaboration. We routinely participate in idea discussions and visit collaborator's laboratories. Bruce Buchholz, who is the principal point of contact for our collaborators, visits

various researchers at the University of California, Davis on a routine, bi-monthly basis. Other resource staff have visited or hosted visitors from: University of California, Davis; University of California, San Francisco; Harvard University; Oregon State University; Georgetown University; Rutgers University; Kansas State University; NIH; and FDA to mention a few. Additionally, we have been encouraging new collaborations with private industry, with visits to: Amgen, Genentech, Merck, Novartis, Picarro Inc., and AnaBios. These visits usually involve one- on- one conversations with collaborators as well as seminars to disseminate information to a wider audience. Specific examples of seminars can be found in the dissemination component.

4) In- person visits to the resource:

New users visit the Resource to receive training on experimental design and data interpretation, as well as observing and understanding the preparation and measurement of samples. Training begins with the Resource's training manual and associated test. These continue to be a key tool, used to train investigators working in the Resource's laboratories and have proven to be a good way to verify a new user's knowledge of standard AMS policies, practice and procedures. Learning radioisotope handling techniques and sample preparation methods follows this foundational knowledge.

5) Postdoctoral scientists and students

Postdoctoral scientists and students directly or indirectly associated with the Resource have learned the technology and it applications and taken it with them as they develop their careers, either at LLNL or elsewhere.

Postdoctoral Researchers include:

Dr. Daniel McCartt, previously a graduate student with the group, has been hired as a postdoc

Dr. Sean Gilmore, previously funded by a LLNL-UC Davis Cancer Center Fitzpatrick Fellowship, is now a LLNL postdoc

Dr. Benjamin Siddoway, postdoc in neuroscience at The Scripps Research Institute

Dr. Windy McNerney, a neurobiology postdoc

Dr. Omran Abu Aboud, a UC Davis postdoc, spends 30-40% of his time at LLNL

Dr. Tiffany Scharadin, UC Davis, drug resistance

Dr. Maike Zimmerman, UC Davis/AMD Joint Fellowship, drug resistance

Dr. King Sin Lee, UC Davis, receptor hunting

Dr. Helen Chow, MD, UC Davis Medical School Resident

Dr. Ketan Patel, University of Leicester, Leukemia diagnostic investigations

Students associated with the Resource:

Erin Madeen, Oregon State University

Swaroop Mistry, UC Merced

Gwendolyn Kaeser, UC San Diego

Sarah Carratt, UC Davis

Training future new users of the resource can start by convincing students interested in science to pursue a career in biology. The Resource provides over 50 tours each year to visiting students, teachers, scientists, scientific leaders, and other interested members of the public. These tours highlight the applications of AMS to biological problems and help inspire current and new generations of scientists. Docents form the LLNL Public Affairs Office escort a weekly public tour with 10-20 guests of the BioAMS facility for which staff answers questions. Every summer 200-300 students from all disciplines in residence at LLNL tour the BioAMS facilities led by AMS staff. Last year, other visitors to the facility included:

Date	Name	Affiliation
11-May-15	Nick Merrill	Waters Corporation
17-Jun-15	Richard Madden	University of Chicago
17-Jun-15	Regan Dunn	University of Wyoming
24-Jun-15	Michael Daly	Waters Corporation
10-Jul-15	COSMOS students	UC Davis
28-Jul-15	High school Teachers and Students	Tracy Unified School District
30-Jul-15	California Council on Science and Technology Policy Fellows	California State Senate and Legislature

14-Aug-15	Local Science Teachers	LLNL Science Education Proram
2-Sep-15	Andy Weir	author
21-Sep-15	Dr. Geraldine Richmond	University of Oregon
21-Sep-15	Dr. Nora Berrah	University of Connecticut
23-Sep-15	Wouter Halswijk	TNO - The Netherlands Organization for Applied Scientific Research
24-Sep-15	Michael Lucchesi	Waters Corporation
13-Oct-15	Dr. Mark Kirk	Department of Homeland Security
14-Oct-15	Scott D. Hammond	UC Santa Barbara Translational Medicine Research Laboratories
19-Oct-15	Michael Daly	Waters Corporation
29-Oct-15	Lucio Calcagnile	University of Salento
29-Oct-15	Gianluca Quarta	University of Salento
29-Oct-15	Silvia Boi	University of Salento
29-Oct-15	Giuseppe Maruccio	University of Salento
29-Oct-15	Andrea Potenza	University of Salento
29-Oct-15	Chiara Gioia	University of Salento
29-Oct-15	Eugenio Elia	University of Salento
29-Oct-15	Angela Aprile	University of Salento
29-Oct-15	Valeria Caggiula	University of Salento
29-Oct-15	Federica Parasporo	University of Salento
29-Oct-15	Maria Vittoria Mastrangelo	University of Salento
16-Dec-15	Brian Rustad	DOE-BES
22-Dec-15	High school Teachers and Students	Stockton Collegiate High School
25-Jan-16	Erin Madeen	Oregon State University
1-Feb-16	Erin Madeen	Oregon State University
16-Feb-16	Local Civic Leaders	Leadership Livermore Group
23-Feb-16	ROTC students	ROTC interns
24-Feb-16	Program Leaders	US Customs and Border Protection
21-Mar-16	Sarah Carratt	UC Davis
24-Mar-16	Dr. Kin Sing Lee	UC Davis

Resource staff has an established track record in outreach; mentoring summer interns, high school and middle school teachers, and giving presentations at the local high schools, community colleges and to the public. Examples of Resource

Outreach include:

ACS Science Coach Activity
February 19, 2015

Bruce Buchholz is taking part in the newly established Science Coach Program offered through the American Chemical Society. He works directly with 5th grade teacher, John Moran from Del Rey Elementary School in Orinda, CA. Together, their goal is to enhance the students' science education by connecting the Common Core science curriculum concerning the water cycle and basic chemistry to the environment around their school. Following a short lesson in the classroom examining a model watershed that represented the area around the school, each 5^{th} grade class at the school tested the water quality in the surrounding creeks. They used test strip kits to measure standard water quality parameters. They tested for nitrates, nitrites, phosphates, iron, hardness and pH. The students then compiled the data between the three participating classes. With the help of their science coach, they are analyzing the data and will present their findings to the "Friends of Orinda Creeks", a local community organization whose mission is to conserve, restore, and educate the public about the values of and threats to the local creeks.

Mentored Brittney Murray, Army cadet summer student

Mentored Allison Guilderson, Science Teacher and Researcher (STAR)

See Also “Overall B.4” and the Dissemination Component for more information about specific training activities associated with each component.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

See Overall B.4.pdf

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? Not Applicable
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? NOTHING TO REPORT
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B DRAFT

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:									**Total Senior/Key Person**	

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: Dissemination
Component Project Lead Information: Kulp, Kristen S

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Dissemination is an important part of this Resource; AMS is unique technology and requires effort to inform researchers of its capabilities and availability. Goals for dissemination include increasing: publications in peer reviewed journals; presentations at conferences and symposia/workshops; and sponsored workshops to deliver information about Biomedical AMS to the research community. Patents are also a form of dissemination and LLNL is particularly keen on transferring technology to the commercial sector so that commercial products eventually become more broadly available.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: dissemination progress.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: See Overall B4.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

See B.2 "accomplisments"

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Future plans for dissemination include: Organizing a session on AMS at a national meeting; planning a Users Workshop that focuses on the versatility of the liquid sample interface; establishing an international AMS Users group (G. Bench, B. Buchholz) that will include quarterly video conferences to share user experiences; presentations (oral or via posters) of research involving AMS at Research Institutions, including NIH, and at National/International meetings; and targeting review articles to popular journals and news magazines via LLNL press releases.

Dissemination is an important part of this Resource; AMS is a relatively unique technology and does require effort to inform researchers of its capabilities and availability. This reporting period, dissemination of information on AMS has been accomplished through a combination of publications, lectures, and press releases. Many of these are featured on our website, (https://bioams.llnl.gov/), under the "What's New" tab. Specific activities are described below for each component. A "resource bibliography" is appended below as well as in the Administration Accomplishments.

Overall:
LLNL BioAMS Article makes Editor's List for Editorial Board of *Chemical Research in Toxicology* favorite CRT Article.
Every year at the fall ACS meeting, the Editorial Board of Chemical Research in Toxicology meets to enjoy a social hour and dinner. At the 2015 meeting in Boston, the Editorial Advisory Board members were asked to pick their favorite paper published in CRT in the past two years; these would be collected in a virtual issue. 16 papers were highlighted and chosen. This paper was one of the 16: *Human in Vivo Pharmacokinetics of [^{14}C]Dibenzo[def,p]chrysene by Accelerator Mass Spectrometry Following Oral Microdosing* Erin Madeen, Richard A. Corley, Susan Crowell, Kenneth Turteltaub, Ted Ognibene, Mike Malfatti, Tammie J. McQuistan, Mary Garrard, Dan Sudakin, and David E. Williams *Chem. Res. Toxicol.*, 2015, **28** (1): 126–134. PMCID: PMC4303324. DOI: 10.1021/tx5003996

Research Resource scientist Bruce Buchholz appeared with collaborators Kirsty Spalding and Jonas Frisén of the Karolinska Institute in Stockholm, Sweden on the episode "Elements" of the National Public Radio program Radiolab. In a segment on the element carbon the trio discussed how they developed radiocarbon bomb pulse dating to determine the age of cells in the human body. The program was broadcast in August 2015. A podcast of the program can be downloaded at http://www.radiolab.org/tags/carbon/.

Wired science writer Sarah Zhang featured the trio of Research Resource scientist Bruce Buchholz, Kirsty Spalding and Jonas Frisén of the Karolinska Institute in Stockholm in a feature on radiocarbon bomb pulse dating that appeared online on March 11, 2016. The article is available at http://www.wired.com/2016/03/obviously-dont-set-off-nukes-cool-science-obviously/.

Participated in a Symposium at the 250th American Chemical Society Meeting in Boston, MA organized by DBP6 PI Paul Henderson. The symposium entitled, "New Approaches to the Study of Chemical Toxicology in Human Health: Accelerator Mass Spectrometry," featured oral presentations from resource staff and collaborators.

TR&D1:
Results were disseminated in publications and presentations.

- Critical Role of Histone Turnover in Neuronal Transcription and Plasticity extended bomb pulse dating to a specific protein and appeared in *Neuron* (Maze et al, 2015).

- Alterations in tryptophan metabolism in renal cell carcinoma were reported in *Cancer Research* (Wettersten et al, 2015).

- Dr. Nima Etminan presented several talks on turnover of structural proteins of aneurysms and cerebral arteries and the clinical implications.

TR&D2:
Results were disseminated in publications and presentations.

- A new approach to surface labeling of nanoparticles followed by biodistribution studies appeared in *Nanoscale* (Nallathamby et al, 2015).
- Evidence of a nonlinear dose response for the protective effects of resveratrol in cancer chemoprevention appeared in *Science Translational Medicine* (Cai et al, 2015).
- The effect of Paclitaxel enhancing Carboplatin-DNA adduct formation and cytotoxicity appeared *Chemical Research in Toxicology* (Jiang et al, 2015).
- An investigation of platinum resistance in a bladder cancer cell culture appeared in *PLoS ONE* (Wang et al, 2016).
- The disposition of a dietary mutagen is described in healthy and pancreatic cancer compromised people in *Chemical Research in Toxicology* (Malfatti et al, 2016).
- Carlos Valdez, Timothy Carpenter, Brian Bennion Michael Malfatti, Heather Enright, Nicholas Be, Windy McNerney, Tuan Nguyen and Felice Lightstone. Development and optimization of a blood-brain barrier permeable AChE deactivator. DTRA Advanced and Emerging Threat Division, Project-Level Science and Technology Review. July 20-24 2015.
- Turteltaub KW, Bench G, Ognibene T, Malfatti M, Loots G, Buchholz B, Enright H. Biomedical applications of accelerator mass spectrometry: Past, present, and future, *American Chemical Society Fall Meeting*, August 17, 2015, Boston, MA
- Enright H, Falso M, Lao V, Malfatti M, Kuhn E, Hum N, Shi Y, Haack K, Kulp K, Buchholz BA, Loots G, Bench G, Turteltaub K. Perinatal exposure to triclocarban results in altered lipid metabolism, *American Chemical Society Fall Meeting*, August 17, 2015, Boston, MA
- Enright H, Malfatti M, Buchholz B, Ognibene T, Turteltaub K. Assessing Responses to Environmentally Relevant Doses Using Accelerator Mass Spectrometry, *International Society of Exposure Science*, October 2015, Henderson, NV

- Turteltaub K, Enright H, Malfatti M, Wheeler E, Carpenter T. Novel Blood Brain Barrier Models to Assess PhIP transport into the Brain. Society of Toxicology Annual Meeting, New Orleans, LA. March 12-17, 2016.

TR&D 3:

Dissemination of TR&D 3 during this performance period consisted of two publications and 3 presentations.

- A description of the interface to couple small liquid samples to AMS appeared in *Nuclear Instruments and Methods in Physics Research B (NIMB)* (Ognibene et al, 2015).
- AN overview of small bioAMS spectrometers appeared in in *Nuclear Instruments and Methods in Physics Research B (NIMB)* (Ognibene et al, 2015).

- A presentation on the use of the liquid sample AMS interface was presented at the NIGMS P41 PI Meeting.
- A presentation on the use of the interface for human tracing was given at Pittcon 2016.
- A general seminar was presented at UC Davis, Department of Biomedical Engineering Seminar Series, October 29, 2015.

DBP1:
Nothing to report

DBP2:
Nothing to report

DBP3:

- Dr. Kin Sing Lee presented a poster at Pacifichem 2015 entitled, “Chemoenzymatic synthesis of epoxy-docosapentaeoic acids.”
- Dr. Kin Sing Stephen Lee presented a seminar entitled, “Investigating the Mechanism on how Drug-Target Residence Time (tR) Affects in vivo Efficacy of Drug with Soluble Epoxide Hydrolase,” at the Dept. of Pharmacology and Toxicology, Michigan State University (February 15, 2016).
- Dr. Kin Sing Stephen Lee presented a seminar entitled, “Investigating the Mechanism on how Drug-Target Residence Time (tR) Affects in vivo Efficacy of Drug with Soluble Epoxide Hydrolase” at the Biology and Biotechnology Division, Lawrence Livermore National Laboratory (March 24, 2016).

DBP4:
Nothing to report

DBP5:
There were several dissemination activities resulting from the analysis of samples from humans exposed to Dibenzo(*def,p*)chrysene (DBC):

- A poster was presented at the Gordon Research Conference on Drug Metabolism entitled, “Environmentally Relevant Microdosing of Human Volunteers For PAH Pharmacokinetics.” (July 17, 2015)
- A poster was presented at OSU Environmental & Molecular Toxicology Research Day entitled, “Estimated Dietary Exposure to Benzo[a]pyrene (BaP) During Clinical Dietary Restriction.” (January 21, 2016)

DBP6:
DBP6 was disseminated to the public at several scientific meetings and forums. Papers involving DBP6 were included with TR&D2.

- Maike Zimmermann, Sisi Wang, Tzu-yin Lin, Hongyong Zhang, Michael Malfatti, David R. Gandara, Marc Dall’Era, George Cimino, James Keck, Ralph de Vere White, Kenneth W. Turteltaub, Chong-xian Pan and Paul T. Henderson. Poster entitled “Personalized Chemotherapy through the Combination of Microdosing and Accelerator Mass Spectrometry”. The Association for Mass Spectrometry: Applications to the Clinical Lab (MSACL) Annual Meeting, San Diego, CA, March 28-April 1, 2015.
- Paul T. Henderson, Oral presentation titled “Use of radiocarbon-labeled cytotoxic drugs for precision medicine applications in cancer therapy.” 250th National Meeting of the American Chemical Society, Boston, MA titled “New Approaches to the Study of Chemical Toxicology in Human Health: Accelerator Mass Spectrometry” presented August 17, 2015.
- Tiffany Scharadin, Hongyong Zhang, Maike Zimmermann, Sisi Wang, Michael Malfatti, George Cimino, Chong-xian Pan, Paul T. Henderson, poster presentation titled “ Quantification of gemcitabine incorporation into DNA to determine drug sensitivity and mechanisms of resistance in bladder cancer cells” presented at the 250th National Meeting of the American Chemical Society, Boston, MA on August 17, 2015.
- Paul T. Henderson, Oral presentation titled “Quantitation of DNA adducts formed by deoxynucleoside analogues and alkylating agents by accelerator mass spectrometry”
- 12th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, Princeton NJ, on June 7-11, 2015
- Paul T. Henderson, Oral presentation titled “Carboplatin diagnostic microdosing trial: Overview of regulatory program and issues of relevance”
- Frontiers in Microdosing for Clinical and Diagnostic Development Symposium, Seoul National University, Seoul, South Korea, on August 18, 2015.
- Paul T. Henderson, Oral presentation titled “Use of radiocarbon labeled drugs for precision medicine applications in cancer therapy”

- Frontiers in Microdosing for Clinical and Diagnostic Development Symposium, Seoul National University, Seoul, South Korea, on August 18, 2015.
- This talk was also presented the same week at the Korean Institute of Science and Technology, Chungnam University and two companies, Biocore and Curachem.

DBP7:
DBP7 was disseminated through a poster presentation.

- Nicholas Hum, Kelly Martin, Deepa Murugesh, Kurt W. Haack, Bruce A. Buchholz, Gabriela G. Loots. Development of Quantitative Methods for Assessing Metastatic Potential of Human Primary Tumors. UC Davis Comprehensive Cancer Center, 21st Annual Cancer Research Symposium. Sacramento, CA. October 30, 2015.

CP1:
Nothing to report.

CP2:
Nothing to report

CP3:

- Nima Etminan, Unravelling the age of the physiological and pathological cerebrovasculature, Joint meeting, American and German Academy for Neurosurgery, Heidelberg, Germany, October 10, 2015.
- .

CP4:
This research has been presented at conferences during the reporting period.

Conferences:

American Chemical Society
Date: August 2015
Title: Perinatal exposure to triclocarban results in altered lipid metabolism
Authors: Enright H, Falso M, Lao V, Malfatti M, Kuhn E, Hum N, Shi Y, Haack K, Kulp K, Buchholz B, Loots G, Bench G, Turteltaub KW**.**

International Society of Exposure Science
Date: October 2015
Title: Assessing Responses to Environmentally Relevant Doses Using Accelerator Mass Spectrometry
Authors: Enright H, Malfatti M, Buchholz B, Ognibene T, Turteltaub K**.**

CP5:
Nothing to report

CP6:
Work was disseminated with 1 publication and 3 presentations.

- McCartt, A. D., Ognibene, T., Bench, G., & Turteltaub, K. (2015). Measurements of carbon-14 with cavity ring-down spectroscopy. *NUCLEAR INSTRUMENTS AND METHODS IN PHYSICS REASEARCH B*, 361:10–13.
- McCartt, A.D., (2015, June 19th) *Measurements of Carbon-14 with Cavity Ring-Down Spectroscopy*. LLNL Post-doc Poster Symposium, Livermore, CA
- McCartt, A.D., (2015, June 19th) *Laser-Based Measurements of ^{14}C.* Presentation, LLNL CAMS, Livermore, CA
- McCartt, A.D., (2015, August 11th) *Laser-Based Measurements of ^{14}C.* Status Update, Picarro, Santa Clara, CA

CP7:
A paper was published.

Hudson BD, Hum NR, Thomas CB, Kohlgruber A, Sebastian A, Collette NM, Coleman MA, Christiansen BA, Loots GG. SOST Inhibits Prostate Cancer Invasion. PLoS One. 2015 Nov 6;10(11):e0142058. doi: 10.1371/journal.pone.0142058. eCollection 2015. PMCID: PMC4636315

RESOURCE BIBLIOGRAPHY

Peer-reviewed Publications:

1. Cai H, Scott E, Kholghi A, Andreadi C, Rufini A, Karmokar A, Britton RG, Horner-Glister E, Greaves P, Jawad D, James M, Howells L, Ognibene T, Malfatti M, Goldring C, Kitteringham N, Walsh J, Viskaduraki M, West K, Miller A, Hemingway D, Steward WP, Gescher AJ, Brown K. Cancer chemoprevention: Evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice. Sci Transl Med. 2015 Jul 29;7(298): 298ra117. doi: 10.1126/scitranslmed.aaa7619. PMID: 26223300 [PubMed - in process]
2. Hooper-Bui LM, Kwok ES, Buchholz BA, Rust MK, Eastmond DA, Vogel JS. Insecticide Transfer Efficiency and Lethal Load in Argentine Ants. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:665-669. PMCID: PMC4615608
3. Hudson BD, Hum NR, Thomas CB, Kohlgruber A, Sebastian A, Collette NM, Coleman MA, Christiansen BA, Loots GG. SOST Inhibits Prostate Cancer Invasion. PLoS One. 2015 Nov 6;10(11):e0142058. doi: 10.1371/journal.pone.0142058. eCollection 2015. PMCID: PMC4636315
4. Jiang S, Pan AW, Lin TY, Zhang H, Malfatti M, Turteltaub K, Henderson PT, Pan CX. Paclitaxel Enhances Carboplatin-DNA Adduct Formation and Cytotoxicity. Chem Res Toxicol. 2015 Dec 21;28(12):2250-2. doi: 10.1021/acs.chemrestox.5b00422 PMID: 26544157 [PubMed - in process]
5. Malfatti MA, Kuhn EA, Turteltaub KW, Vickers SM, Jensen EH, Strayer L, Anderson K. The disposition of the dietary mutagen 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline in healthy and pancreatic cancer compromised humans. Chem Res Toxicol. 2016 Feb 26. [Epub ahead of print]. PMID: 26918625 [PubMed - as supplied by publisher]
6. Maze I, Wenderski W, Noh KM, Bagot RC, Tzavaras N, Purushothaman I, Elsässer SJ, Guo Y, Ionete C, Hurd YL, Tamminga CA, Halene T, Farrelly L, Soshnev AA, Wen D, Rafii S, Birtwistle MR, Akbarian S, Buchholz BA, Blitzer RD, Nestler EJ, Yuan ZF, Garcia BA, Shen L, Molina H, Allis CD. Critical Role of Histone Turnover in Neuronal Transcription and Plasticity. Neuron. 2015 Jul 1;87(1):77-94. doi: 10.1016/j.neuron.2015.06.014. PMCID: PMC4491146
7. McCartt, A. D., Ognibene, T., Bench, G., & Turteltaub, K. (2015). Measurements of carbon-14 with cavity ring-down spectroscopy. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:10–13.
8. Nallathamby PD, Mortensen NP, Palko HA, Malfatti M, Smith C, Sonnett J, Doktycz MJ, Gu B, Roeder RK, Wang W, Retterer ST. New surface radiolabeling schemes of super paramagnetic iron oxide nanoparticles (SPIONs) for biodistribution studies. Nanoscale 2015 Apr 21;7(15):6545-55. doi: 10.1039/c4nr06441k. PMID: 25790032 [PubMed - indexed for MEDLINE]
9. Ognibene TJ, Haack KW, Bench G, Brown TA, & Turteltaub KW. Operation of the ,"Small" BioAMS spectrometers at CAMS: Past and future prospects. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:54-57. PMCID: PMC4594839

10. Ognibene TJ, Thomas AT, Daley PF, Bench G, & Turteltaub KW. An interface for the direct coupling of small liquid samples to AMS. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:173-177. PMCID: PMC4594830
11. Tooker BC, Brindley SM, Chiarappa-Zucca ML, Turteltaub KW, Newman LS. Accelerator mass spectrometry detection of beryllium ions in the antigen processing and presentation pathway. J Immunotoxicol. 2015 Apr-Jun;12(2):181-7. doi: 10.3109/1547691X.2014.917748. Epub 2014 Jun 16. PubMed PMID: 24932923; PubMed Central PMCID: PMC4426990.
12. Wang S, Zhang H, Scharadin TM, Zimmermann M, Hu B, Pan AW, Vinall R, Lin TY, Cimino G, Chain P, Vuyisich M, Gleasner C, Mcmurry K, Malfatti M, Turteltaub K, de Vere White R, Pan CX, Henderson PT. Molecular Dissection of Induced Platinum Resistance through Functional and Gene Expression Analysis in a Cell Culture Model of Bladder Cancer. PLoS One. 2016 Jan 22;11(1):e0146256. doi: 10.1371/journal.pone.0146256. eCollection 2016. PMCID: PMC4723083
13. Wettersten HI, Hakimi AA, Morin D, Bianchi C, Johnstone ME, Donohoe DR, et al. Grade-Dependent Metabolic Reprogramming in Kidney Cancer Revealed by Combined Proteomics and Metabolomics Analysis. Cancer Res. 2015;75(12):2541-52. Epub 2015/05/09. doi: 10.1158/0008-5472.CAN-14-1703. PubMed PMID: 25952651; PubMed Central PMCID: PMC4470795

Conference Abstracts and Presentations:

1. Turteltaub K, Bench G, Ognibene T, Malfatti M, Loots G, Buchholz B, Enright H. Biomedical Applications of Accelerator Mass Spectrometry: Past, present, and future. 250th National Meeting of the American Chemical Society, Boston, MA. August 17, 2015.
2. Turteltaub K, Enright H, Malfatti M, Wheeler E, Carpenter T. Novel Blood Brain Barrier Models to Assess PhIP transport into the Brain. Society of Toxicology Annual Meeting, New Orleans, LA. March 12-17, 2016.
3. Valdez C, Carpenter T, Bennion B, Malfatti M, Enright H, Be N, McNerney W, Nguyen T, Lightstone F. Development and optimization of a blood-brain barrier permeable AChE deactivator. DTRA Advanced and Emerging Threat Division, Project-Level Science and Technology Review. Washington, DC. July 20-24 2015.
4. Ted Ognibene. Applications of Accelerator Mass Spectrometry to Human Health Research. Pittsburgh Conference on Analytical, Chemistry and Applied Spectroscopy. Atlanta, GA. March 6-10, 2016. Invited Talk.
5. Benjamin J. Stewart. Direct Coupling of HPLC-MS with Accelerator Mass Spectrometry for ^{14}C-labeled Analyte Measurement. NIGMS P41 PI Meeting. Rockville, MD. March 14, 2016.
6. Madeen EP, Ognibene TJ, McQuistan TJ, Turteltaub KW, Williams DE. Environmentally Relevant Microdosing of Human Volunteers For PAH

Pharmacokinetics. Gordon Research Conference on Drug Metabolism. Holderness, NH, July 12-17, 2015.

7. Nicholas Hum, Kelly Martin, Deepa Murugesh, Kurt W. Haack, Bruce A. Buchholz, Gabriela G. Loots. Development of Quantitative Methods for Assessing Metastatic Potential of Human Primary Tumors. UC Davis Comprehensive Cancer Center, 21st Annual Cancer Research Symposium. Sacramento, CA. October 30, 2015.
8. Kin Sing Stephen Lee, Jun Yang, Lionel Cheruzel, Bruce Hammock, Ashly Lewis. Chemoenzymatic synthesis of epoxy-docosapentaeoic acids. Pacifichem 2015. Honolulu, HI. December 15-20, 2015. Poster.
9. Maike Zimmermann, Sisi Wang, Tzu-yin Lin, Hongyong Zhang, Michael Malfatti, David R. Gandara, Marc Dall'Era, George Cimino, James Keck, Ralph de Vere White, Kenneth W. Turteltaub, Chong-xian Pan and Paul T. Henderson. Poster entitled "Personalized Chemotherapy through the Combination of Microdosing and Accelerator Mass Spectrometry". The Association for Mass Spectrometry: Applications to the Clinical Lab (MSACL) Annual Meeting, San Diego, CA, March 28-April 1, 2015.
10. Paul T. Henderson, Oral presentation titled "Use of radiocarbon-labeled cytotoxic drugs for precision medicine applications in cancer therapy." 250th National Meeting of the American Chemical Society, Boston, MA titled "New Approaches to the Study of Chemical Toxicology in Human Health: Accelerator Mass Spectrometry" presented August 17, 2015.
11. Tiffany Scharadin, Hongyong Zhang, Maike Zimmermann, Sisi Wang, Michael Malfatti, George Cimino, Chong-xian Pan, Paul T. Henderson, poster presentation titled " Quantification of gemcitabine incorporation into DNA to determine drug sensitivity and mechanisms of resistance in bladder cancer cells" presented at the 250th National Meeting of the American Chemical Society, Boston, MA on August 17, 2015.
12. Paul T. Henderson, Oral presentation titled "Quantitation of DNA adducts formed by deoxynucleoside analogues and alkylating agents by accelerator mass spectrometry"
13. 12th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, Princeton NJ, on June 7-11, 2015.
14. Enright H, Falso M, Lao V, Malfatti M, Kuhn E, Hum N, Shi Y, Haack K, Kulp K, Buchholz BA, Loots G, Bench G, Turteltaub K. Perinatal exposure to triclocarban results in altered lipid metabolism, *American Chemical Society Fall Meeting*, Boston, MA. August 17, 2015.
15. Carratt SA, Buchholz BA, Ding X, Van Winkle LS. Naphthalene DNA Adduct Formation in Ex-Vivo Rodent Tissues. Society of Toxicology Meeting, New Orleans, LA. March 14, 2016.
16. Buchholz BA, Beckett L, Turteltaub KW, Bova GS. Cancer Metastasis Aging by Carbon 14 Dating. UC Davis Cancer Center Research Symposium, Sacramento, CA. October 2015.

17. Mortimer M, Petersen EJ, Buchholz BA, Orias E, Holden PA. Separation of Bacteria, Protozoa and Carbon Nanotubes by Density Gradient Centrifugation. Emerging Contaminants Summit, Westminster, CO. March 1-2, 2016.
18. Enright H, Malfatti M, Buchholz B, Ognibene T, Turteltaub K. Assessing Responses to Environmentally Relevant Doses Using Accelerator Mass Spectrometry, *International Society of Exposure Science*, October 2015, Henderson, NV.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

See Overall B.4.pdf

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-8633

RESEARCH & RELATED BUDGET - SECTION A & B **DRAFT**

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
Total Funds Requested for all Senior Key Persons in the attached file												
Additional Senior Key Persons:		File Name:								**Total Senior/Key Person**		

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**0.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**0.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**0.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name:
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. COMPONENT COVER PAGE

Project Title: Administration
Component Project Lead Information: Turteltaub, Kenneth W

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The enduring goals of the resource are: to demonstrate methods for the routine use of AMS in biomedical research, to expand measurement capabilities and capacities, to increase collaborative access to the instrument, and to provide analytical services, while training others to use and expand AMS in biomedical research. The goals of the administration component are to: provide for the overall administration, management and fiscal oversight of the Resource; prioritize and streamline the sample analysis for external collaborative/service projects; facilitate the training of investigators; and disseminate the capabilities of the Resource. It plays a central role in the integration of the TR&Ds, DBPs and Collaborative projects.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: administration accomplishments 2016.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: See Overall B4.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

See Dissemination Component

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

The future plans of the administrative component are to continue to provide for the overall administration, management and fiscal oversight of the Resource. A major challenge for the coming year will be to prioritize the efforts of the Resource-supported investigators to maximize scientific productivity given the mandated 10% reduction in budget. A second goal will be to further explore the operational space of the new HPLC- BioAMS instrument to include a greater variety of elution conditions compatible with the liquid sample interface. A third goal will be to expand the user base of the resource.

Administration Accomplishments

The administration section of the Resource for Biomedical AMS provided for the overall administration, management and fiscal oversight of the Resource. It played a central role in the integration of the TR&Ds, DBPs and Collaborative projects. A significant accomplishment of the administration component was the purchase of two new analytical instruments: a Waters Xevo G2 XS QTOF instrument for qualitative and quantitative sample analysis and an Elementar BiovisION IRMS instrument for quantifying $^{13}C/^{12}C$ isotope ratios as well as CHN elemental analysis. Together, the addition of these two instruments increases the analytical capabilities of the laboratory and increases the breadth of information we can obtain from our samples.

1. Describe the administration structure of the resource

This Resource has been in operation since 1999 using the same operational and business model. It is a mature organization that has fostered the growth of AMS in the biosciences and supported over 90 collaborators and Service users, overseen the publication of 150+ research articles, trained postdocs and students, and overseen the development of the AMS technology now available through our Resource and other adopting groups. The staff and facilities of the Resource sit within the Center for Accelerator Mass Spectrometry (CAMS) and the Biosciences and Biotechnology Research Division (BBTD). Both belong to the Physical and Life Sciences Directorate (PLS). PLS supports the Laboratory's science, technological, and programmatic missions in life sciences. The directorate contributes to the rapidly expanding science of biology in areas relevant to important challenges in national security, environmental sustainability, and human health. Integrating bioscience and biotechnology, PLS performs research in the areas of genome biology, computational biology, molecular toxicology, host- pathogen biology, biochemical structures, assays, genetics and microbial systems, and the intersection of these areas with advances in nanotechnology, and imaging and measurement science. Scientists working within PLS form a core of expertise that supports the Resource. In addition to the oversight provided by the LLNL infrastructure, an internal Executive Committee and external national Advisory Committee will oversee the Resource activities.

The overall management and administration of the Resource is provided by Dr. Kenneth Turteltaub (PI) and Dr. Graham Bench (Co-PI). These investigators have worked together for approximately 20 years and led the original establishment of this Center. The administration core is comprised of Drs. Turteltaub and Bench, Dr. Kristen Kulp, and a resource administrator. Dr. Kulp is the Group Leader for the Pharmacology and Toxicology Group within BBTD and has over 10 years experience managing large projects and mentoring staff. Dr. Kulp and the administrator work closely with the PI, project investigators, laboratory staff, and various collaborators to ensure smooth operation of the Resource. Dr. Turteltaub is the primary contact; he coordinates all aspects of the process for access to the Resource by external and internal investigators. Dr. Bruce Buchholz is the primary contact for the collaborators for scientific issues within the projects. Dr. Buchholz is a radiochemist with 20 years experience using AMS in his research and has worked with many of our collaborators to design experiments, troubleshoot and interpret data. Other Resource staff and members of the CAMS staff also provide scientific guidance to the DBPs and collaborative/service projects, as appropriate.

The PI and Co-PI consult on regular basis with the Resource staff through the Internal Executive Committee meetings and with the National Advisory Board who reviews the research completed, research proposed, and provides assistance in developing future directions. This BioAMS group meets weekly on Wed mornings for 90 minutes and has done so for the last 15 years.

Resource Operating Procedures

Decisions regarding operation of the Resource will be determined under the general guidelines of the Biotechnology Resources Technology Program at NIH and receive input from our National Advisory Committee. Resource operations include management and professional development, collaborations with external investigators, and outreach activities to educate the user community on AMS.

Resource management, including long range planning, policy implementation, fiscal management, daily prioritization, and ensuring efficient operations are the responsibility of the Internal Executive Committee consisting of K. Turteltaub, G. Bench, K. Kulp. M. Malfatti, G. Loots, T. Ognibene, B. Buchholz and B. Stewart. Meetings of the Executive Committee for project updates and administrative matters are held weekly. Proposal Review Panel meetings to review applications for access to the Resource are scheduled and held as needed. This panel consists of the Internal Executive Committee members and members of the CAMS staff who offer additional AMS expertise of value to specific proposals on an ad-hoc basis when needed.

Standardized procedures have been developed to insure sample tracking from all studies and to insure rapid return of the data to internal and external collaborative/service investigators. Samples are logged in upon receipt and are assigned a tracking number upon submission for AMS analysis. A complete set of SOPs for all aspects of AMS analysis, including sample receipt and storage, equipment use and maintenance in bio labs, Lab procedures, Data handling and Reporting and Record retention has been created and is updated as needed.

Submission of Proposals for Access to the Biomedical AMS Research Resource

Initial contact with the Resource happens in many different ways but usually begins with an email or phone call from a potential collaborator or service user who has either read a publication using AMS with our contact information, reviewed our website, or been referred to us from a collaborator, service user, or other colleague. After initial discussion on the feasibility of the project and the need for AMS relative to other approaches, a visit or expanded conference call is arranged where the project is discussed in more detail. Once a project has been designed the collaborator or service user applies for formal access. In order to obtain access to Resource services, all biomedical researchers will be requested to submit an application, which includes a formal proposal or a letter of intent regarding their scientific problem. A specific explanation of the study requirements is required on the “Application for Access to the NIH Resource for Biomedical AMS” form. Proposals and letters of intent will be able to be submitted at any time. These forms are available at our website https://bioams.llnl.gov/

The operational procedures for access to the Resource are available to the public on the Resource website at http://bioams.llnl.gov/. Prior to formally applying for access to the Resource, an investigator must discuss a proposal concept with an appropriate

Resource staff member for evaluation of project compatibility with AMS. Only after pre-screening is an application for access to the Resource submitted to the Resource Administrator. This process has served to eliminate the need to reject applications. Resource members play a key role in the project, according to their areas of research interest and expertise.

As a condition for use of the Biomedical AMS Resource, it is expected that investigators will pursue publication of their project's results within one year of successfully completing the studies. When using data in publications and/or presentations, they must acknowledge NIH Resource for Biomedical Accelerator Mass Spectrometry and the supporting NIH/NIGMS grant funding. In compliance with NIH policy, all publications are made publically available. Additionally, copies of preprints for review and release by LLNL and reprints are to be submitted to the Resource Administrator.

IACUC AND IRB COMPLIANCE

The Research Resource and its subprojects are in compliance with the Lawrence Livermore National Laboratory's Institutional Animal Care and Use Committee (IACUC) guidelines as well as the Institutional Review Board (IRB) requirements.
The requisite approvals are on file and copies of the approval letters are available upon request.

2. Awards and Honors

Ken Turteltaub was named a fellow of the American Association for the Advancement of Science (AAAS) in November 2015. Turteltaub was specifically cited for the development of ultra-precise accelerator mass spectrometry (AMS) methods for biomedicine and work on carcinogenesis, the formation of macromolecules and low-dose pharmacokinetics.

3. Advisory Committee

External National Advisory Committee

The External National Advisory Committee is in place to oversee the Resource's scientific direction and operating policies. In addition, it comments on the needs and plans for adequate instrumentation, technology development, and future scientific direction. It helps recruit investigators for collaboration in new areas of research. The makeup of the committee was weighted by expertise in mass spectrometry to help guide the needs in technology development. It was also weighted by expertise in carcinogenesis and pharmacology/toxicology since these had been the theme areas for the Resource during its initial funding period. The membership of the External National Advisory Committee is currently being reviewed to ensure that the external expertise reflects the current scientific focus areas. A.L Burlingame, Ph.D., a senior investigator at University of California at San Francisco and expert in biomedical applications of mass spectrometry, has continued to serve as Chairman of the Committee. The members of the committee are listed in Table 1 below.

Table 1. Resource National Advisory Board Membership and Expertise

Committee Member	***Institution***	***Expertise***
B. Hammock	University of California, Davis	Cancer Etiology/ Pharmacology/ Toxicology
T. A. Baillie	University of Washington	Mass Spectrometry /Drug Metabolism
A. L. Burlingame	University of California, San Francisco	Biological Mass Spectrometry
J. T. Brenna	Cornell University	Nutrition/ Mass Spec Interfaces
James Felton	University of California, Davis	Toxicology
J. Davis	LLNL/ Hertz Foundation	Accelerator Mass Spectrometry
I. Cristea	Princeton University	Molecular Biology Proteomics

Internal Executive Committee

The Internal Executive Committee members are from the Resource's key personnel list. The Internal Executive Committee, along with Resource staff and associated members of the Center for Accelerator Mass Spectrometry, meet weekly to discuss project effort and prioritization, review collaborative/service requests, and deal with other issues relating to Biomedical AMS. The members of the committee are listed below.

Member's Name	**Department**
Kenneth W. Turteltaub, Ph.D. Principal Investigator	Biosciences & Biotechnology Division
Graham Bench, Ph.D. Co- Principal Investigator	Center for Accelerator Mass Spectrometry
Bruce A. Buchholz, Ph.D.	Center for Accelerator Mass Spectrometry
Heather Enright, Ph.D.	Biosciences & Biotechnology Division
Mike Malfatti, Ph.D.	Biosciences & Biotechnology Division
Ted J. Ognibene, Ph.D.	Center for Accelerator Mass Spectrometry
Kristen S. Kulp, Ph.D.	Biosciences & Biotechnology Division
Gabriela Loots, Ph.D.	Biosciences & Biotechnology Division
Benjamin Stewart, Ph.D.	Biosciences & Biotechnology Division

The External Advisory Committee, in conjunction with the Internal Executive Committee, strives to meet once a year. Additionally, Partial Committee meetings can occur on *ad hoc* occasions, such as workshops. Discussions also occur as needed by phone, email, or fax. The Annual Advisory Committee Meeting focuses on the progress being made on the Technology Development projects, the allocation of resources, and the Resource's future.

4. External Advisory Committee report

Nothing to report. The External Advisory Committee did not meet during the past 12 months of the grant funding.

5. Patents, Licenses, Inventions, and Copyrights
Nothing to report.

6. Personnel Roster

	Name	**Degree**	**Department**	**Institution**
Overall	Kenneth Turteltaub	PhD	Biosciences and Biotechnology	LLNL, CA
	Graham Bench	PhD	Center for Accelerator Mass Spectrometry	LLNL, CA
	Kristen Kulp	PhD	Biosciences and Biotechnology	LLNL, CA
	Ed Kuhn	BS	Biosciences and Biotechnology	LLNL, CA
	Kurt Haack	BS	Biosciences and Biotechnology	LLNL, CA
TRD1	Bruce Buchholz	PhD	Center for Accelerator Mass Spectrometry	LLNL, CA
	Benjamin Stewart	PhD	Biosciences and Biotechnology	LLNL, CA
TRD2	Michael Malfatti	PhD	Biosciences and Biotechnology	LLNL, CA
TRD3	Ted Ognibene	PhD	Biosciences and Biotechnology	LLNL, CA
DBP1	Kirsty Spalding	PhD	Cell and Molecular Biology	Karolinska Institute, Sweden
DBP2	Alma Burlingame	PhD	Mass Spectrometry Center	University of California, San Francisco
	Giselle Knudsen	PhD	Mass Spectrometry Center	University of California, San Francisco
DBP3	Bruce Hammock	PhD	Entomology	University of California, Davis
	Kin Sing Lee	PhD	Entomology	University of California, Davis
DBP4	David Baker	PhD	Institute for Protein Design	University of Washington

	Ingrid Pultz	PhD	Institute for Protein Design	University of Washington
DBP5	David Williams	PhD	Environmental and Molecular Toxicology	Oregon State University
	Erin Madeen	Grad student	Environmental and Molecular Toxicology	Oregon State University
	Sharon Krueger	PhD	Linus Pauling Institute	Oregon State University
	Tammie McQuistan	BSc	Linus Pauling Institute	Oregon State University
	Sandra Uesugi	RN, MSc	Linus Pauling Institute	Oregon State University
DBP6	Paul Henderson	PhD	Internal Medicine	University of California, Davis
	Chong-xian Pan	MD, PhD	Internal Medicine	University of California, Davis
	Maike Zimmermann	PhD	Internal Medicine	University of California, Davis
	Tiffany Scharadin	PhD	Internal Medicine	University of California, Davis
	Brian Jonas	MD, PhD	Internal Medicine	University of California, Davis
	Helen Chow	MD	Internal Medicine	University of California, Davis
	Hongyong Zhang	DVM, PhD	Internal Medicine	University of California, Davis
	Tzuyin Lin	DVM, PhD	Internal Medicine	University of California, Davis
	Karim Chamie	MD	Urology	University of California, Los Angeles
	Max Meng	MD	Urology	University of California, San Francisco
	George Cimino	PhD		Accelerated Medical Diagnostics, CA
DBP7	Gabriela Loots	PhD	Biosciences and Biotechnology	LLNL, CA
	Ervin Epstein	MD	Dermatology	Children's Hospital Oakland Research, CA
	Grace Wang	PhD		Children's Hospital Oakland Research, CA
CP1	Kit Lam	MD PhD	Biochemistry and Molecular Medicine	University of California, Davis
	Wenwu Xiao	PhD	Biochemistry and Molecular Medicine	University of California, Davis
CP2	Donald Vander	PhD	Surgery	University of

	Griend			Chicago, IL
CP3	Nima Etminan	MD	Neurosurgery	Heidelberg University, Mannheim, Germany
	Rita Dreier	PhD	Institute for Physiological Chemistry and Pathobiochemistry	Westfalian Wilhelms-University, Münster, Germany
	Daniel Cooke	MD	Radiology	University of California, San Francisco
	Steven Hetts	MD	Neurointervention Radiology	University of California, San Francisco
	Helen Kim	PhD	Anesthesia	University of California, San Francisco
	Michael Lawton	MD	Neurological Surgery	University of California, San Francisco
	Hua Su	MD	Anesthesia	University of California, San Francisco
	R. Loch Macdonald	MD, PhD	Neurosurgery	St. Michael's Hospital, Toronto Canada
	R. Loch Macdonald	MD, PhD	Neurosurgery	University of Toronto, Toronto Canada
CP4	Heather Enright	PhD	Biosciences and Biotechnology	LLNL, CA
	Nicholas Hum	MS	Biosciences and Biotechnology	LLNL, CA
	Yilan Shi	MS		Bio-Rad, CA
CP5	Karen Brown	PhD	Cancer Studies	University of Leicester, Leicester, UK
	Martin Dyer	MB BCh, DPhil	Cancer Studies	University of Leicester, Leicester, UK
CP6	Alan McCartt	PhD	Center for Accelerator Mass Spectrometry	LLNL, CA
	Christopher Rella	PhD		Picarro, CA
Other collab.	Robert Weiss	MD	Internal Medicine	University of California, Davis
	Omran Aboud	DVM, PhD	Internal Medicine	University of California, Davis
	Patricia Holden	PhD	Environmental Science	University of

			and Management	California, Santa Barbara
	Elijah Petersen	PhD	Biosystems and Biomaterials	NIST, MD
	David Allis	PhD	Chromatin Biology and Epigenetics	The Rockefeller University, NY
	Ian Maze	PhD	Chromatin Biology and Epigenetics	The Rockefeller University, NY
	John Casida	PhD	Environmental Science, Policy and Management	University of California, Berkeley
	Laura Van Winkle	PhD	Center for Health and the Environment	University of California, Davis
	Sarah Carratt	Grad student	Center for Health and the Environment	University of California, Davis
	Karen Bales	PhD	Psychology	University of California, Davis
	Trish Berger	PhD	Animal Science	University of California, Davis
	Alan Conley	PhD	Population Health and Reproduction	University of California, Davis
	Jerold Chun	PhD	Molecular Biology	Scripps, CA
	Ben Siddoway	PhD	Molecular Biology	Scripps, CA
	Gwendolyn Kaeser	Grad student	Molecular Biology	University of California, San Diego
	Alan Buckpitt	PhD	Molecular Biosciences	University of California, Davis
	K. Alkass	PhD	Forensic Medicine	Karolinska Institute Sweden
	Lindsay Allen	PhD	Western Human Nutrition Research Center	USDA, CA
	Steven Bova	MD	Sidney Kimmel Comprehensive Cancer Center	Johns Hopkins, MD
	Peter Bruckner	PhD	Institute for Physiological Chemistry	University Hospital of Munster, Munster, Germany
	Andrew Clifford	PhD	Nutrition	University of California, Davis
	Richard Corley	PhD	Biological Sciences	Pacific Northwest National Laboratory, WA
	Michael Dennison	PhD	Environmental Toxicology	University of California, Davis
	Henrik Druid	PhD, MD	Forensic Medicine	Karolinska Institute Sweden
	Jonas Frisen	PhD, MD	Cell and Molecular Biology	Karolinska Institute Sweden
	Marjorie Garrod	BS	Medical Pathology	University of California, Davis

	Shirley Gee	MS	Entomology	University of California, Davis
	Ralph Green	MD	Medical Pathology	University of California, Davis
	Daniel Hanggi	PhD	Neurosurgery	Heinrich-Heine University, Germany
	Joshua Miller	PhD	Nutritional Sciences	Rutgers University, NJ
	Monika Mortimer	PhD	Bren School of Environmental Science and Management	University of California, Santa Barbara
	Daniel Murnick	PhD	Physics	Rutgers University, NJ
	Robert Rice	PhD	Environmental Toxicology	University of California, Davis
	Setti Shabab-Ferdows	PhD	Western Human Nutrition Research Center	USDA, CA
	Miranda Sarachine-Falso	PhD	Biology	Slippery Rock University, PA
	Hans-Jacob Steiger	PhD	Neurosurgery	Heinrich-Heine University, Germany
	Ralph DeVere White	MD	Urology	University of California, Davis
	Jason Cannon	PhD	Health Sciences and Toxicology	Purdue University, IN
	Marina Chiarappa-Zucca	PhD	Materials Science Division	LLNL, CA
	Nick Hum	MS	Biosciences and Biotechnology Division	LLNL, CA
	Jun Yang	PhD	Entomology	University of California, Davis
	Richard Madden	PhD	Evolutionary Biology	University of Chicago
Advisory Board	Thomas Baillie	PhD	Pharmacy	University of Washington, WA
	Thomas Brenna	PhD	Human Ecology	Cornell University, NY
	Jay Davis	PhD		Hertz Foundation, CA
	James Felton	PhD	Biosciences and Biotechnology	LLNL-retired, CA
	Ilena Cristea	PhD	Molecular Biology	Princeton University, NJ

7. Investigator Support

a) **Non-Federal & Federal-Non PHS**

Investigator Name: Gabriela Loots
Title: Characterizing Host-Pathogen Immunity-Gut-Brain Interactions
Organization: LLNL/DOE
Grant/Contract Number: LDRD-16-ERD-007 (started)
Funds: $550,000
Subproject: TR&D2, DBP7

Investigator Name: Gabriela Loots
Title: Optimizing Drug Efficacy through Pharmacogenomics-Driven Personalized Therapy
Organization: LLNL/DOE
Grant/Contract Number: LDRD-13-ERD-042 (closed)
Funds: $550,000
Subproject: TR&D2, DBP7

Investigator Name: Ted Ognibene
Title: PLS-Revolutionizing AMS Throughput
Organization: DOE/ISCP
Grant/Contract Number: 39590
Funds: $350,000/ yr
Subproject: TR&D3

Investigator Name: R. Weiss/ B.Stewart
Title: Targeting pH regulation through inhibition of glutamine metabolism as a novel therapeutic approach for renal cell carcinoma (RCC)
Organization: LLNL-UCDCC Fitzpatrick Award
Grant/Contract Number:
Funds: $50,000
Subproject: Collaborative Project

Investigator Name: C Pan/P Henderson
Title: Use of AMS method to analyze DNA damage/repair kinetics of ABT-888 + carboplatin in tumor cells
Organization: Abbott Laboratories
Grant/Contract Number: UCD Project #201121855
Funds: $79,122
Subproject: DBP6

Investigator Name: C Pan
Title: Development of targeting nanotherapeutics against bladder cancer
Organization: VA Merit.
Grant/Contract Number: 1I01BX001784
Funds: $650,000
Subproject: DBP6, CP1

Investigator Name: Kit Lam
Title: Multifunctional nanoporphyrin against bladder cancer
Organization: DoD PRMRP
Grant/Contract Number: W81XWH-13-1-0490
Funds: $2,270,000
Subproject: TR&D2, CP1

Investigator Name: D. Vander Griend
Title: FUNCTION AND CLINICAL UTILITY OF THE HOX PROTEIN CO-FACTORS MEIS1 AND MEIS2
Organization: DOD
Grant/Contract Number: PC130587
Funds: $225,000
Subproject: CP2

Investigator Name: D Cooke
Title: The role of neural stem cells in the response-to-injury paradigm of brain AVM pathogenesis
Organization: UCSF Seed Grant
Grant/Contract Number: Unknown
Funds: Unknown
Subproject: CP3

Investigator Name: Karen Brown
Title: Quantitative analysis of cell turnover: a strategy for personalising treatment of chronic lymphocytic leukaemia.
Organization: Leukaemia and Lymphoma Research (UK charity)
Grant/Contract Number: Unknown
Funds: $100,000
Subproject: CP5

Investigator Name: Karen Brown
Title: Cancer stem cell analysis
Organization: National Institute for Health Research (UK)
Grant/Contract Number: Unknown
Funds: $143,000/yr
Subproject: CP5

Investigator Name: Buchholz
Title: Mechanism for Clastrogenic Activity of Naphthalene
Organization: DoD/CDMRP/LCRP
Grant/Contract Number: LC130820
Funds: $100,000
Subproject: other collaborative

b) **Federal- PHS Support**

Investigator Name: Kin Sing Lee
Title: Identifying the Receptors of Environmentally Sensitive Epoxy-Eicosanoids with AMS
Organization: NIH/NIEHS
Grant/Contract Number: K99 ES024806-1
Funds: unknown
Subproject: DBP3

Investigator Name: David Williams
Title: Comparative Mechanisms of Cancer Chemoprevention
Organization: NIH, NCI
Grant/Contract Number: P01 CA90890-09
Funds: $235,000
Subproject: DBP5

Investigator Name: David Williams
Title: Flavin-Containing Monooxygenase in Lung
Organization: NIH, NHLBI
Grant/Contract Number: R01 HL038650-25
Funds: $250,000
Subproject: DBP5

Investigator Name: R. Weiss
Title: METABOLOMIC DISCOVERY AND VALIDATION OF URINARY BIOMARKERS FOR KIDNEY CANCER
Organization: NIH
Grant/Contract Number: 5R01CA135401-05
Funds: $300,000
Subproject: collaborative project

Investigator Name: B. Hammock
Title: Hydrolytic enzymes in the metabolism of toxins
Organization: NIH/NIEHS
Grant/Contract Number: R01 ES002710
Funds: $289,692
Subproject: DBP3

Investigator Name: B. Hammock
Title: Biomarkers of exposure to hazardous substances,
Organization: NIH/NIEHS
Grant/Contract Number: P42 ES04699
Funds: $1,597,225
Subproject: DBP3, Collaborative projects

Investigator Name: KS Lee
Title: Chemoprevention of pancreatic Carcinogenesis via targeting c-RAF and sEH
Organization: NIH/NCI
Grant/Contract Number: R01 CA172431
Funds: $37,196 to UC Davis
Subproject: DBP3

Investigator Name: P Henderson
Title: Phase 0 Microdosing Diagnostics Clinical Trial
Organization: NIH/NCI
Grant/Contract Number: SBIR Contract HHSN261201200048C
Funds: $750,000
Subproject: TR&D2, DBP6

Investigator Name: P Henderson
Title: Colorectal Cancer Oxaliplatin Microdosing Trial
Organization: NIH/NCI
Grant/Contract Number: SBIR Contract HHSN261201200048C
Funds: $100,000
Subproject: TR&D2, DBP6

Investigator Name: P Henderson
Title: Characterization of ErbB Receptors in Nanoparticles
Organization: NIH/NCI
Grant/Contract Number: R01CA155642
Funds: $201,275
Subproject: TR&D2, DBP6

Investigator Name: Kit Lam
Title: Therapeutic targeting agents for ovarian cancer
Organization: NIH/NCI
Grant/Contract Number: R01CA115483
Funds: $217,137
Subproject: TR&D2, CP1

Investigator Name: Kit Lam
Title: Multi-Functional Nanocarrier Against Canine Lymphoma
Organization: NIH/NIBIB
Grant/Contract Number: R01EB012569
Funds: $499,245
Subproject: CP1

Investigator Name: C Pan
Title: CANCER CENTER SUPPORT GRANT
Organization: NCI
Grant/Contract Number: 2 P30 CA0933730 – 09

Funds: $15,000
Subproject: DBP6

Investigator Name: Paul Henderson
Title: Nanoparticles to eradicate leukemia stem cells
Organization: NCI SBIR
Grant/Contract Number: unknown
Funds: $200,000
Subproject: DBP6

Investigator Name: D Vander Griend
Title: FUNCTION OF THE STEM CELL TRANSCRIPTION FACTOR SOX2 IN PROSTATE CANCER
Organization: NIH/NCI
Grant/Contract Number: 1R01CA178431-01A1
Funds: $1,036,500
Subproject: CP2

Investigator Name: D Cooke
Title: Career support to foster clinical and basic science stroke research
Organization: NIH/NINDS
Grant/Contract Number: 1U10NS086494-01
Funds: Unknown
Subproject: CP3

Investigator Name: K Turteltaub
Title: Development of Laser Spectroscopic Methods For Quantification of ^{14}C
Organization: NIH/NIGMS
Grant/Contract Number: R21GM111242
Funds: $234,368
Subproject: CP6

Investigator Name: Buchholz
Title: Role of Microfibrils in the Biology of Pathology of the Eye
Organization: NIH/NEI
Grant/Contract Number: 1R01EY024607-01
Funds: $11,400 to LLNL
Subproject: other collaborative

Investigator Name: Buchholz
Title: Metabolic Mechanisms of Naphthalene Toxicity in Lung
Organization: NIH/NIEHS
Grant/Contract Number: 3 R01 ES020867-02S1
Funds: $250,000
Subproject: other collaborative

Investigator Name: Chun

Title: Single Cell sequencing and in situ mapping of RNA transcripts in human brains
Organization: NIH/NIMH
Grant/Contract Number: U01 MH098977
Funds: $200,000/yr
Subproject: other collaborative

8. Summary Tables

a) Subprojects

	TR&D	DBPs	C&S	Training	Dissemination
# of Subprojects	**3**	**7**	**19**	**NA**	**NA**
# of Investigators	**5**	**26**	**59**	**NA**	**NA**
# Number of Publications	**7**	**3**	**3**	**NA**	**NA**

b) Geographical usage

Foreign investigators by country

Country	Number of Investigators
Canada	1
Germany	5
Sweden	4
United Kingdom	2

U.S. investigators by state

State	Number of Investigators
California	67
Illinois	2
Indiana	1
Maryland	3
New Jersey	3
New York	3
Oregon	5
Pennsylvania	1
Washington	3

c) Publications

i. journal publications: 13
ii. book chapters: 0
iii. abstracts: 17

d) Investigator support

i. non-federal support- total dollars $1,022,122

Agency	Amount	Number of Investigators
Abbott Laboratories	79,122	2
VA Merit	650000	4
Leukaemia and Lymphoma Research (UK charity)	100000	2
National Institute for Health Research (UK)	143000	2
LLNL-UCDCC Fitzpatrick Award	50000	1
Totals	1,022,122	11

ii. non-PHS federal support- total dollars $3,495,000

Agency	Amount	Number of Investigators
DOE/ISCP	350,000	1
LLNL/DOE	550,000	3
DoD PRMRP	2,270,000	3
DoD	225,000	1
DOD/CDMRP/LCRP	100,000	3
Totals	3,495,000	11

Iii PHS support- total dollars $6,174,038

Agency	Amount	Number of Investigators
HHSN	850,000	6
CA	2,042,108	11
EB	449,245	2
GM	234,368	2
HL	250,000	3
EY	11,400	1
MH	200,000	3
ES	2,136,917	6
Totals	6,174,038	34

RESOURCE BIBLIOGRAPHY

Peer-reviewed Publications:

1. Cai H, Scott E, Kholghi A, Andreadi C, Rufini A, Karmokar A, Britton RG, Horner-Glister E, Greaves P, Jawad D, James M, Howells L, Ognibene T, Malfatti M,

Goldring C, Kitteringham N, Walsh J, Viskaduraki M, West K, Miller A, Hemingway D, Steward WP, Gescher AJ, Brown K. Cancer chemoprevention: Evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice. *Sci Transl Med*. 2015 Jul 29;7(298): 298ra117. doi: 10.1126/scitranslmed.aaa7619. PMID: 26223300 [PubMed - in process]

2. Hooper-Bui LM, Kwok ES, Buchholz BA, Rust MK, Eastmond DA, Vogel JS. Insecticide Transfer Efficiency and Lethal Load in Argentine Ants. *Nucl Instrum Methods Phys Res B*. 2015 Oct 15;361:665-669. PMCID: PMC4615608
3. Hudson BD, Hum NR, Thomas CB, Kohlgruber A, Sebastian A, Collette NM, Coleman MA, Christiansen BA, Loots GG. SOST Inhibits Prostate Cancer Invasion. *PLoS One*. 2015 Nov 6;10(11):e0142058. doi: 10.1371/journal.pone.0142058. eCollection 2015. PMCID: PMC4636315
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Conference Abstracts and Presentations:

1. Turteltaub K, Bench G, Ognibene T, Malfatti M, Loots G, Buchholz B, Enright H. Biomedical Applications of Accelerator Mass Spectrometry: Past, present, and future. 250th National Meeting of the American Chemical Society, Boston, MA. August 17, 2015.
2. Turteltaub K, Enright H, Malfatti M, Wheeler E, Carpenter T. Novel Blood Brain Barrier Models to Assess PhIP transport into the Brain. Society of Toxicology Annual Meeting, New Orleans, LA. March 12-17, 2016.
3. Valdez C, Carpenter T, Bennion B, Malfatti M, Enright H, Be N, McNerney W, Nguyen T, Lightstone F. Development and optimization of a blood-brain barrier permeable AChE deactivator. DTRA Advanced and Emerging Threat Division, Project-Level Science and Technology Review. Washington, DC. July 20-24 2015.
4. Ted Ognibene. Applications of Accelerator Mass Spectrometry to Human Health Research. Pittsburgh Conference on Analytical, Chemistry and Applied Spectroscopy. Atlanta, GA. March 6-10, 2016. Invited Talk.
5. Benjamin J. Stewart. Direct Coupling of HPLC-MS with Accelerator Mass Spectrometry for ^{14}C-labeled Analyte Measurement. NIGMS P41 PI Meeting. Rockville, MD. March 14, 2016.
6. Madeen EP, Ognibene TJ, McQuistan TJ, Turteltaub KW, Williams DE. Environmentally Relevant Microdosing of Human Volunteers For PAH Pharmacokinetics. Gordon Research Conference on Drug Metabolism. Holderness, NH, July 12-17, 2015.
7. Nicholas Hum, Kelly Martin, Deepa Murugesh, Kurt W. Haack, Bruce A. Buchholz, Gabriela G. Loots. Development of Quantitative Methods for Assessing Metastatic Potential of Human Primary Tumors. UC Davis

Comprehensive Cancer Center, 21st Annual Cancer Research Symposium. Sacramento, CA. October 30, 2015.

8. Kin Sing Stephen Lee, Jun Yang, Lionel Cheruzel, Bruce Hammock, Ashly Lewis. Chemoenzymatic synthesis of epoxy-docosapentaeoic acids. Pacifichem 2015. Honolulu, HI. December 15-20, 2015. Poster.
9. Maike Zimmermann, Sisi Wang, Tzu-yin Lin, Hongyong Zhang, Michael Malfatti, David R. Gandara, Marc Dall'Era, George Cimino, James Keck, Ralph de Vere White, Kenneth W. Turteltaub, Chong-xian Pan and Paul T. Henderson. Poster entitled "Personalized Chemotherapy through the Combination of Microdosing and Accelerator Mass Spectrometry". The Association for Mass Spectrometry: Applications to the Clinical Lab (MSACL) Annual Meeting, San Diego, CA, March 28-April 1, 2015.
10. Paul T. Henderson, Oral presentation titled "Use of radiocarbon-labeled cytotoxic drugs for precision medicine applications in cancer therapy." 250th National Meeting of the American Chemical Society, Boston, MA titled "New Approaches to the Study of Chemical Toxicology in Human Health: Accelerator Mass Spectrometry" presented August 17, 2015.
11. Tiffany Scharadin, Hongyong Zhang, Maike Zimmermann, Sisi Wang, Michael Malfatti, George Cimino, Chong-xian Pan, Paul T. Henderson, poster presentation titled " Quantification of gemcitabine incorporation into DNA to determine drug sensitivity and mechanisms of resistance in bladder cancer cells" presented at the 250th National Meeting of the American Chemical Society, Boston, MA on August 17, 2015.
12. Paul T. Henderson, Oral presentation titled "Quantitation of DNA adducts formed by deoxynucleoside analogues and alkylating agents by accelerator mass spectrometry" 12th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, Princeton NJ, on June 7-11, 2015.
13. Enright H, Falso M, Lao V, Malfatti M, Kuhn E, Hum N, Shi Y, Haack K, Kulp K, Buchholz BA, Loots G, Bench G, Turteltaub K. Perinatal exposure to triclocarban results in altered lipid metabolism, *American Chemical Society Fall Meeting*, Boston, MA. August 17, 2015.
14. Carratt SA, Buchholz BA, Ding X, Van Winkle LS. Naphthalene DNA Adduct Formation in Ex-Vivo Rodent Tissues. Society of Toxicology Meeting, New Orleans, LA. March 14, 2016.
15. Buchholz BA, Beckett L, Turteltaub KW, Bova GS. Cancer Metastasis Aging by Carbon 14 Dating. UC Davis Cancer Center Research Symposium, Sacramento, CA. October 29, 2015.
16. Mortimer M, Petersen EJ, Buchholz BA, Orias E, Holden PA. Separation of Bacteria, Protozoa and Carbon Nanotubes by Density Gradient Centrifugation. Emerging Contaminants Summit, Westminster, CO. March 1-2, 2016.
17. Enright H, Malfatti M, Buchholz B, Ognibene T, Turteltaub K. Assessing Responses to Environmentally Relevant Doses Using Accelerator Mass

Spectrometry, *International Society of Exposure Science*, October 2015, Henderson, NV.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

See Overall B.4.pdf

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B **DRAFT**

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Kenneth		Turteltaub		Project Lead	276,483.00	2.4			48,127.00	28,440.00	76,567.00
2.		Graham		Bench		Co-Lead	238,902.00	2.4			40,917.00	24,182.00	65,099.00
3.		Bruce		Buchholz		Project Investigator	155,460.00	1.8			20,502.00	12,116.00	32,618.00
4.		Heather		Enright		Project Investigator	110,459.00	2.4			18,918.00	11,181.00	30,099.00
5.		Gabriela		Loots		Project Inestigator	155,460.00	1.8			20,502.00	12,116.00	32,618.00
6.		Michael		Malfatti		Project Investigator	140,642.00	1.2			12,044.00	7,118.00	19,162.00
7.		Ted		Ognibene		Project Investigator	171,600.00	1.8			22,630.00	13,374.00	36,004.00
8.		Benjamin		Stewart		Project Investigator	126,787.00	1.2			10,857.00	6,417.00	17,274.00
9.		Kris		Kulp		Project Investigator	212,953.00	1.2			18,236.00	10,778.00	29,014.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **338,455.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Project Staff	8.4			60,587.00	35,807.00	96,394.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**96,394.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**434,849.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	10,100.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**10,100.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 827171463

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF CALIF-LAWRNC LVRMR NAT LAB

Start Date*: 06-01-2016 **End Date*:** 05-31-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	94,738.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Other AMS analysis related expenses	281,790.00
Total Other Direct Costs	**376,528.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**821,477.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Facilicities and Administrative Costs	78.5	821,478.00	644,870.00
		Total Indirect Costs	**644,870.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DOE Livermore Site Office, David Goett, 925-422-8217

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,466,347.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget justification.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification

No significant changes from the previous budget are proposed. The personnel, supplies and other expenses are the same as last year.

Public Access Compliance

Grant Number:	5P41GM103483-18
PD/PI Name:	Turteltaub, Kenneth W.
Project Title:	Resource for the Development of Biomedical Accelerator Mass Spectrometry (AMS)
PRAM submitted on:	
File Uploaded:	PRAM_for_Ticket25329.pdf

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Tooker BC, Brindley SM, Chiarappa-Zucca ML, Turteltaub KW, Newman LS. Accelerator mass spectrometry detection of beryllium ions in the antigen processing and presentation pathway. J Immunotoxicol. 2015 Apr-Jun;12(2):181-7. doi: 10.3109/1547691X.2014.917748. Epub 2014 Jun 16. PubMed PMID: 24932923; PubMed Central PMCID: PMC4426990.
Complete	Griggs AM, Agim ZS, Mishra VR, Tambe MA, Director-Myska AE, Turteltaub KW, McCabe GP, Rochet JC, Cannon JR. 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is selectively toxic to primary dopaminergic neurons in vitro. Toxicol Sci. 2014 Jul;140(1):179-89. doi: 10.1093/toxsci/kfu060. Epub 2014 Apr 9. Erratum in: Toxicol Sci. 2014 Oct;141(2):560. PubMed PMID: 24718704; PubMed Central PMCID: PMC4133585.
Complete	Ognibene TJ, Haack KW, Bench G, Brown TA, Turteltaub KW. Operation of the "Small" BioAMS Spectrometers at CAMS: Past and Future Prospects. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:54-57. Epub 2015 May 26. PubMed PMID: 26456990; PubMed Central PMCID: PMC4594839.
Complete	Hooper-Bui LM, Kwok ES, Buchholz BA, Rust MK, Eastmond DA, Vogel JS. Insecticide Transfer Efficiency and Lethal Load in Argentine Ants. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:665-669. PubMed PMID: 26504258; PubMed Central PMCID: PMC4615608.
Complete	Jiang S, Pan AW, Lin TY, Zhang H, Malfatti M, Turteltaub K, Henderson PT, Pan CX. Paclitaxel Enhances Carboplatin-DNA Adduct Formation and Cytotoxicity. Chem Res Toxicol. 2015 Dec 21;28(12):2250-2. doi: 10.1021/acs.chemrestox.5b00422. Epub 2015 Nov 11. PubMed PMID: 26544157; PubMed Central PMCID: PMC4834887.
Complete	Tong T, Ondov JM, Buchholz BA, VanDerveer MC. Contemporary carbon content of bis (2-ethylhexyl) phthalate in butter. Food Chem. 2016 Jan 1;190:1064-8. doi: 10.1016/j.foodchem.2015.06.053. Epub 2015 Jun 20. PubMed PMID: 26213077; PubMed Central PMCID: PMC4816072.
Complete	Wang S, Zhang H, Scharadin TM, Zimmermann M, Hu B, Pan AW, Vinall R, Lin TY, Cimino G, Chain P, Vuyisich M, Gleasner C, Mcmurry K, Malfatti M, Turteltaub K, de Vere White R, Pan CX, Henderson PT. Molecular Dissection of Induced Platinum Resistance through Functional and Gene Expression Analysis in a Cell Culture Model of Bladder Cancer. PLoS One. 2016 Jan 22;11(1):e0146256. doi: 10.1371/journal.pone.0146256. eCollection 2016. PubMed PMID: 26799320; PubMed Central PMCID: PMC4723083.

Complete	Malfatti MA, Kuhn EA, Turteltaub KW, Vickers SM, Jensen EH, Strayer L, Anderson KE. Disposition of the Dietary Mutagen 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline in Healthy and Pancreatic Cancer Compromised Humans. Chem Res Toxicol. 2016 Mar 21;29(3):352-8. doi: 10.1021/acs.chemrestox.5b00495. Epub 2016 Mar 8. PubMed PMID: 26918625; PubMed Central PMCID: PMC4831706.
Complete	Hudson BD, Hum NR, Thomas CB, Kohlgruber A, Sebastian A, Collette NM, Coleman MA, Christiansen BA, Loots GG. SOST Inhibits Prostate Cancer Invasion. PLoS One. 2015 Nov 6;10(11):e0142058. doi: 10.1371/journal.pone.0142058. eCollection 2015. PubMed PMID: 26545120; PubMed Central PMCID: PMC4636315.
Complete	Nallathamby PD, Mortensen NP, Palko HA, Malfatti M, Smith C, Sonnett J, Doktycz MJ, Gu B, Roeder RK, Wang W, Retterer ST. New surface radiolabeling schemes of super paramagnetic iron oxide nanoparticles (SPIONs) for biodistribution studies. Nanoscale. 2015 Apr 21;7(15):6545-55. doi: 10.1039/c4nr06441k. PubMed PMID: 25790032.
Complete	Cai H, Scott E, Kholghi A, Andreadi C, Rufini A, Karmokar A, Britton RG, Horner-Glister E, Greaves P, Jawad D, James M, Howells L, Ognibene T, Malfatti M, Goldring C, Kitteringham N, Walsh J, Viskaduraki M, West K, Miller A, Hemingway D, Steward WP, Gescher AJ, Brown K. Cancer chemoprevention: Evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice. Sci Transl Med. 2015 Jul 29;7(298):298ra117. doi: 10.1126/scitranslmed.aaa7619. PubMed PMID: 26223300; PubMed Central PMCID: PMC4827609.
Complete	Wettersten HI, Hakimi AA, Morin D, Bianchi C, Johnstone ME, Donohoe DR, Trott JF, Aboud OA, Stirdivant S, Neri B, Wolfert R, Stewart B, Perego R, Hsieh JJ, Weiss RH. Grade-Dependent Metabolic Reprogramming in Kidney Cancer Revealed by Combined Proteomics and Metabolomics Analysis. Cancer Res. 2015 Jun 15;75(12):2541-52. doi: 10.1158/0008-5472.CAN-14-1703. Epub 2015 May 7. PubMed PMID: 25952651; PubMed Central PMCID: PMC4470795.
Complete	McCartt AD, Ognibene T, Bench G, Turteltaub K. Measurements of Carbon-14 With Cavity Ring-Down Spectroscopy. Nucl Instrum Methods Phys Res B. 2015 Oct 15;361:277-280. PubMed PMID: 27065506; PubMed Central PMCID: PMC4822718.