Signature Page
Academic Research Council
(ARC) Grant Application

Gabe Gaidos
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P.I. Signature

_____n/a_____

Co-P.I. Signature (if applicable)

George Manacheril
Mon 10/31/2016 4:37 PM
To: Gabriel Gaidos;

I approve Gabe Gaidos's ARC application

← BY EMAIL

Georg Manacheril
Science Dept. Chair

_____

Department Chair or Coordinator

Dean's Sign-off Initials:

i. Method complies with practices of department or division
_____(initial)

ii. Equipment requests are justified and represent needs of department or division._____ (initial)

_____

Signature of Dean

Table of Contents

Introduction/Background

Most of our current food supply comes from industrially processed foods. The benefit of this industrial processing is an abundance of food never before experienced in human history. But the negative side of this trend is that some of the altered foods can be very unhealthy over the long term.

The number of artificial compounds in the food industry is so high that it outstrips regulators' efforts to study their effects. Only compounds causing acute toxicity are screened out, while many, less toxic but still worrisome compounds are commercialized for decades before they are found to be deleterious (ex: BPA in plastics, trans-fats). The FDA maintains a database of food additives called the ***Everything Added to Food in the United States (EAFUS***) database (link). Currently this database has more than 3,000 chemical compounds in it, and the list is growing daily. Consequently, there is a huge need to study food additives in more depth to find out what health risks they might pose.

We will use the fruit fly (*Drosophila melanogaster*) as our model organism because its biology has been extensively characterized for more than 100 years. So many important discoveries have been made on this animal that the list would fill an encyclopedia. Illustrating its importance to current medical research, the fruit fly's genome was sequenced in 2000 by the National Institutes of Health (link). Comparative studies with the human genome have shown that these flies and humans share more than 75% of the disease causing genes (Reiter *et al*, 2001).

Due to its short life span (1-2 months), the effects of accelerated aging, or the effects of decades-long chronic toxicities can be studied in the *'compressed'* life span of the fruit fly. One can find out what ruins a person's health in 50 years by studying the fruit fly for 1 month (link). Compared to their body size, they also eat very large amounts of food – think of having a can of soda the size of a keg. Such high level food consumption amplifies the effects of toxicities they are exposed to.

Finally, the fruit fly is amenable for laboratory work at FSW because it is absolutely harmless, and easy to keep. It also helps that this animal does not fall under animal research regulations. All these properties make the fruit flies one of the favorite experimental animals at most colleges (link).

The flies will be exposed to chemicals and studied as described in the *Method* section.

Goals and Significance

The project's goal is to detect a change in the biochemical markers of health and aging caused by artificial food chemicals (colorants, emulsifiers, taste stabilizers, preservatives, and texture fillers).

The findings hopefully will lead to better foods, which then will lead to gains in health and quality of life. Even if the food industry will be reluctant to change, the information gained will help educate consumers to make healthier choices.

Additionally, FSW students will learn about the biochemical and physiological processes underlying good health. They will learn about the negative effects of processed foods, and they will also learn how to navigate the deceptive advertising environment that surrounds us. Perhaps most importantly, they will have a hands-on experience with the scientific method.

Currently, FSW students do not have access to biochemistry and molecular biology research projects, and the goal would be to fill this gap. I received several requests from students to have such projects (and even one request from a parent!). Interested FSW students will be recruited from the following classes: Human Nutrition (HUN1201), General Chemistry for Health Sciences (CHM2032), Organic Chemistry I and II (CHM2010 and 2011), and Biological Science (BSC1010).

The results will be published in peer-reviewed journals, such as the Journal of Nutrition (JN), or the Journal of Biological Chemistry (JBC), or the Public Library of Science (PLoS). The data will

also be used as *'preliminary data'* in applying for grants from the National Science Foundation (NSF) and the National Institutes of Health (NIH) as described in the *Future Research* section.

Research Questions or Hypothesis

The hypothesis is that certain food chemicals interfere with normal biochemical processes and cause observable negative effects as measured in various biomarkers: telomere length, nuclear morphology, ubiquitination of proteins, and accumulation of advanced glycation end-products.

Method

The basic metabolic processes (such as glycolysis, Krebs cycle, cytochrome P450 detox system) are conserved across many species. This reality is reflected in our DNA, as we share approximately 50% of our genes even with evolutionarily primitive species (worms&insects). As mentioned in the *Introduction*, our chosen model organism is the fruit fly because it shares 75% of disease causing genes with humans.

Data collection:

Students will participate in single-blind and double-blind experimental setups, as to eliminate personal bias, or expectation bias, in data evaluation.

The actual measurable biomarkers **on the cellular level** will be:

- chromosomal telomere lengths: measured by polymerase chain reaction (PCR) assays.
- nuclear membrane morphology: microscope observations and statistical analyses
- levels of ubiquitinated histone proteins H2A/H2B, and levels of advanced glycation end-products (AGEs): measured by Western blot analysis

The measured biomarkers **on the physiological level** will be:

- body morphology, fecundity, and life span of flies: direct observation and statistical analyses.

Selection of Chemicals: As mentioned in the *Introduction* there are over 3,000 food additives currently in use. Therefore it will be important to focus the project on a narrow and hopefully fruitful shortlist of chemicals.

The first *'suspect list'* will be generated by comparing the regulatory lists of other governments (Canada, European Union, Japan, South Korea, Thailand) to the US list. The fact that many other countries already banned compounds that are still legal in the US brings up troubling questions (Calton&Calton 2013). Here is a short list of compounds banned in most developed countries, but still used in the USA:

- Azodicarbonamide - flour bleaching agent
- Butylated Hydroxyanisole (BHA) - preservative for fats
- Butylated Hydroxytoluene (BHT) - preservative for fats
- Blues #1 and #2, Yellows #5 and #6 - colorants
- Brominated triglycerides - emulsifiers
- Olestra - fat substitute
- Potassium bromate - dough texturizer

The second *'suspect list'* will be generated based on how widely they are used, combined with some physiological and biochemical pointers. For example, *caramel* is probably the most widely used additive. It is used as a colorant, flavoring and taste enhancer agent. Unfortunately, the biochemical issue with caramel is that it acts as a seeding agent to generate advanced glycation end-products (AGEs) which are thought to be a major causative agent of aging at the molecular level (Luevano-Contreras 2010, Palimeri *et al* 2015).

Another widely used food additive is *monosodium glutamate (MSG)* which is used as a taste enhancer. In epidemiological studies MSG was statistically linked to obesity (He *et al*, 2011).

A third 'suspect' on this list is *sodium benzoate* which is a widely used preservative - practically all soft drinks contain it. Recently, it was reported that chronic exposure to sodium benzoate could cause glucose metabolism to malfunction (Lennerz *et al*, 2015).

The food chemicals will be purchased in pure form, and mixed in controlled amounts into the food of the experimental animals. Control groups will be set up to study dosage effects of the compounds. Unfortunately, the pricing of the chemicals will also affect the selection process.

Possible conclusions:

We plan to systematically test a long list of food industry compounds. It is very likely that many of these will have no toxicity. It is also quite possible that some food additives may have a BENEFICIAL effect – this would also be a quite valuable piece of information to have.
For those compounds that show a deleterious effect, we will follow up with more focused experiments trying to understand the actual mechanism of toxicity. Such findings in fact will open up new research questions and opportunities for new grants.

Timeline

The following timeline is envisioned during the Spring ’17 semester:
January/February: Drosophila laboratory setup, student recruitment and initial training
February-April: research progresses
May/June: results write-up and preparation for submission of NIH/NSF grant proposals; publication write-up.

Evaluation

Student interest and feedback will be the primary evaluation method. In the long term, the career path of the project’s alumni will also be used to evaluate the usefulness of the project. Additionally, scientific publications and possible new funding will also indicate whether the project is progressing well or not.

Future Research

This project is open ended. Additional ARC funding will be sought in the coming years. Most importantly, the data obtained in this project will be used for submitting grant proposals to the national funding agencies (NSF, NIH) and to private and public foundations as follows:

- The Allen Foundation (link)

- The Nutricia Research Foundation (link)
- The Egg Nutrition Center (link)
- Dannon Institute Nutritional Leadership (link)
- NSF: Improving Undergraduate STEM Education: Education and Human Resources (link)
- NSF: Research at Undergraduate Institutions (RUIs)(link)
- NSF: Career Development Award (link)
- NSF: Scholarships in Science, Technology, Engineering, and Mathematics Program (S-STEM)(link)
- NIH: Food Specific Molecular Profiles and Biomarkers (PAR-15-024)

Budget – see attached budget form

Faculty Member's Background Data

Dr. Gabe Gaidos obtained his Ph.D. in biochemistry in the laboratory of professor Kathrin Kirsch at the Boston University School of Medicine. After graduation he conducted research at Dartmouth College under the guidance of professor Dale Mierke. His research focused on protein biochemistry and on developing drug leads against cancer and viral agents.

Scientific publications:

Identification and Characterization of the Interaction Site between cFLIPL and Calmodulin.
Gaidos G, Panaitiu AE, Guo B, Pellegrini M, Mierke DF.
Public Library of Science 2015 Nov 3;10(11):e0141692. doi: 10.1371/journal.pone.0141692. eCollection 2015.

A novel caspase 8 selective small molecule potentiates TRAIL-induced cell death.
Bucur O, **Gaidos G**, Yatawara A, Pennarun B, Rupasinghe C, Roux J, Andrei S, Guo B, Panaitiu A, Pellegrini M, Mierke DF, Khosravi-Far R.
Scientific Reports 2015 May 11;5:9893. doi: 10.1038/srep09893

Small-molecule inhibitors of JC polyomavirus infection.
Yatawara A, **Gaidos G**, Rupasinghe CN, O'Hara BA, Pellegrini M, Atwood WJ, Mierke DF.
Journal of Peptide Science 2015 Mar;21(3):236-42. doi: 10.1002/psc.2731. Epub 2014 Dec 19.

Gallic acid-based small-molecule inhibitors of JC and BK polyomaviral infection.
O'Hara BA, Rupasinghe C, Yatawara A, **Gaidos G**, Mierke DF, Atwood WJ.
Virus Research 2014 Aug 30;189:280-5. doi: 10.1016/j.virusres.2014.06.008. Epub 2014 Jun 21.

killerFLIP: a novel lytic peptide specifically inducing cancer cell death.
Pennarun B, **Gaidos G**, Bucur O, Tinari A, Rupasinghe C, Jin T, Dewar R, Song K, Santos MT, Malorni W, Mierke D, Khosravi-Far R.
Cell Death and Disease 2013 Oct 31;4:e894. doi: 10.1038/cddis.2013.401.

Structure and function analysis of the CMS/CIN85 protein family identifies actin-bundling properties and heterotypic-complex formation.
Gaidos G, Soni S, Oswald DJ, Toselli PA, Kirsch KH.
Journal of Cell Science. 2007 Jul 15;120(Pt 14):2366-77.

Bibliography

1) He, K., Du, S., Xun, P., Sharma, S., Wang, H., Zhai, F., & Popkin, B. (2011). **Consumption of monosodium glutamate in relation to incidence of overweight in Chinese adults: China Health and Nutrition Survey (CHNS).** The American Journal of Clinical Nutrition, 93(6), 1328–1336. http://doi.org/10.3945/ajcn.110.008870

2) Jayson Calton and Mira Calton (2013) **Rich Food Poor Food: An Eater's Manual** Primal Nutrition, Inc.

3) Lennerz, B., Vafai, S. B., Delaney, N. F., Clish, C. B., Deik, A. A., Pierce, K. A., Mootha, V. K. (2015). **Effects of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans.** Molecular Genetics and Metabolism, 114(1), 73–79. http://doi.org/10.1016/j.ymgme.2014.11.010

4) Luevano-Contreras, C., & Chapman-Novakofski, K. (2010). **Dietary Advanced Glycation End Products and Aging.** *Nutrients*, *2*(12), 1247–1265. http://doi.org/10.3390/nu2121247

5) Palimeri, S., Palioura, E., & Diamanti-Kandarakis, E. (2015). **Current perspectives on the health risks associated with the consumption of advanced glycation end products: recommendations for dietary management.** *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *8*, 415–426. http://doi.org/10.2147/DMSO.S63089

6) Pandey, U. B., & Nichols, C. D. (2011). **Human Disease Models in Drosophila melanogaster and the Role of the Fly in Therapeutic Drug Discovery**. Pharmacological Reviews, 63(2), 411–436. http://doi.org/10.1124/pr.110.003293

7) Reiter, L. T., Potocki, L., Chien, S., Gribskov, M., & Bier, E. (2001). **A Systematic Analysis of Human Disease-Associated Gene Sequences In *Drosophila melanogaster*.** *Genome Research*, *11*(6), 1114–1125. http://doi.org/10.1101/gr.169101

ARC Grant Budget Form

Faculty Name(s): Dr. Gabriel Gaidos

Total Amount Requested: $5,000

Budget Breakdown:

Travel $ n/a

Equipment $3,190

Student Stipend $ n/a (Max $10/hr)

Supplies $1810

Technology/Software $ n/a

Other (Non-equipment) $ n/a

Budget justification. Provide an explanation for all of the expenses you have listed in your budget.

The equipment requested is necessary to set up a basic fruit fly operation.

The Incubators are necessary to house the flies in temperature controlled environment.

The Microscope+Camera+Laptop are necessary for documenting the morphology of the flies.

The Water Bath is necessary for preparing growth media and other solutions.

The Label Maker is necessary to track all bottles, solutions.

The Illuminated Magnifiers are necessary for routine sorting of the flies.

The supplies are specific and general lab consumables to run the experiments.

The food chemicals to be tested.

Growth media for the flies.

DNA oligonucleotides and buffers to run assays.

Pipettes and pipette tips to measure liquids.

Lab coats and goggles.

Disposable gloves.

ARC GRANT EQUIPMENT REQUEST

Gaidos, Gabriel Banner ID: 00365882

Dept: Applied and Pure Sciences

OFFICE/ROOM WHERE EQUIPMENT WILL BE STORED:

Lee, Building H, Room H225

EQUIPMENT TO BE PURCHASED:

ITEM	**COST ESTIMATE**
3.5X-90X LED Zoom Stereo Microscope+5MP Digital Camera	$700
Dell Latitude D630 Laptop for microscope	$120
2 Digital Incubators (2 cubic feet)	$1,600
Drosophila Laboratory Kit	$220
Digital water bath	$400
Label maker	$50
2 Illuminated magnifiers	$100
TOTAL:	**$3,190**