Oregon Bio 2013:
Conference Addendum

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Poster Presentations

Title: Native Fungal Endophytes as Engineers of Novel Agrochemicals

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Daniel J. Ballhorn and Rachel F. Fordyce, Portland State University

In times of a dramatically increasing demand for food and biofuels, sufficient crop yield is one of the greatest challenges worldwide. Similar to human pathogens, plant pathogens and insect pests increasingly develop resistance against pesticides. Thus, there is high pressure to develop new and efficient, yet, ecologically tolerable agrochemicals. In this proposal, we aim to identify the potential of fungal endophytes to produce secondary compounds with bioactivity that can be used as biological and sustainable products for plant protection against both fungal plant pathogens and insect herbivores. Endophytic fungi are a hyperdiverse group of microbes living symptomless in plants. These cryptic organisms are extraordinary biochemical engineers, producing a broad range of natural compounds, some of them being important drugs in cancer treatment such as taxol. Our studies on the native Cascade Oregon Grape (*Mahonia nervosa*) revealed a surprisingly high number of endophytes capable of producing bioactive compounds in vitro with specific activity towards pathogenic fungi and insects. Aims of this project are 1) to isolate, cultivate and identify high numbers of endophytes , 2) prepare extracts from endophyte cultures, 3) to screen extracts for bioactivity against pathogenic fungi and herbivorous insects as well as for unspecific cytotoxity, and 4) to discover ways to develop a commercial product. This project will provide innovative results on the function of endophytes as plant protective agents, it will seek for possibilities to develop sustainable agrochemicals based on endophyte-derived compounds.
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Title: Novel Binding Site Inhibitor Discovery on HIV-1 Matrix Domain

Ayna Alfadhli, Henry McNett, Rachel Sloan, David Peyton and Eric Barklis, Oregon Health & Science University

The matrix domain (MA) of the HIV-1 precursor Gag (PrGag) protein directs PrGag proteins to assembly sites at the plasma membrane by virtue of its affinity to the phospholipid, phosphatidylinositol-4,5-bisphosphate (PI(4,5)P(2)). Additionally, MA has been ascribed other functions such as RNA binding, and facilitating the incorporation of HIV-1 envelope (Env) proteins into virus particles. We have demonstrated that MA binds to RNA at a site that overlaps its PI(4,5)P(2) site, suggesting that RNA binding may protect MA from associating with inappropriate cellular membranes prior to PrGag delivery to the PM. Based on this, we have developed assays in which small molecule competitors to MA-RNA binding can be characterized, with the assumption that such compounds might interfere with essential MA functions and help elucidate additional features of MA binding. Following this approach, we have identified compounds that compete with RNA for MA binding. We also have identified MA residues involved in binding and found that they overlap the MA PI(4,5)P(2) and RNA sites. Cell culture studies demonstrated that these compounds inhibit HIV-1 replication but are associated with significant levels of toxicity. Nevertheless, these observations provide new insights into MA binding and pave the way for the development of antivirals that target the HIV-1 matrix domain.
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Title: Using the Helios NanoLab 650 DualBeam™ for life science applications at OHSU

Claudia S. Lopez[1,3], Jessican L. Riesterer[2] and Eric Barklis[1,3], [1]Oregon Health & Science University, [2]FEI Company, [3]OHSU Multi-Scale Microscopy Core (MMC)
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Title: Blocking IL-1β prevents M2 muscarinic receptor dysfunction in parainfluenza infected guinea pigs.

A.E. Rynko, A.D. Fryer, D.B. Jacoby, Oregon Health & Science University

RATIONALE: Viral infections cause asthma attacks. Viruses cause loss of function of inhibitory M2 receptors on parasympathetic nerves. Blocking IL-1β prevents M2 receptor dysfunction in antigen-challenged guinea pigs. Here we investigated whether blocking IL-1β would prevent M2 receptor dysfunction is virus infected guinea pigs.
METHODS: Guinea pigs were infected with parainfluenza. In some animals, IL-1β was blocked with anakinra (30mg/kg, i.p.) 30min before infection and every 24 hours after infection. Four days after infection, guinea pigs were mechanically ventilated and M2 receptor function was measured as the ability of gallamine to increase vagally stimulated bronchoconstriction. Viral titers were determined by measuring viral RNA in the lungs. White cells in blood and lung lavage were counted.
RESULTS: Anakinra prevented M2 receptor dysfunction in infected animals. White cell counts were increased in lavage and decreased in the blood of infected animals. Anakinra did not affect viral titers or white cell counts.
CONCLUSIONS: Treatment with an IL-1β receptor antagonist prevents M2 receptor dysfunction in parainfluenza infected guinea pigs.
Supported by NIH grants T32 A1074494 (AER), HL61013 (DBJ), AIO92210 (DBJ), ES014601 (ADF), ES017592 (ADF)

Title: Cloudbreak: Accurate and Scalable Genomic Structural Variation Detection in the Cloud with MapReduce

Chris Whelan, Kemal Sonmez and Lucia Carbone, Oregon Health & Science University

The detection of genomic structural variations (SV) remains a difficult challenge in analyzing high-throughput DNA sequencing data, and the growing size and number of sequenced genomes have rendered SV detection a bona fide big data problem, especially as sequencing is increasingly applied at larger scales and in clinical settings. MapReduce is a proven, scalable solution for distributed computing on huge data sets. We describe a conceptual framework for SV detection algorithms in MapReduce based on computing local genomic features, and use it to develop a deletion and insertion detection algorithm, Cloudbreak. On simulated and real data sets, Cloudbreak achieves accuracy improvements over popular SV detection algorithms, and genotypes variants from diploid samples. It provides dramatically shorter runtimes and the ability to scale to big data volumes on large compute clusters. Cloudbreak includes tools to set up and configure MapReduce (Hadoop) clusters on cloud services such as the Amazon Elastic Compute Cloud, enabling on-demand cluster computing. Our implementation and source code are available at http://github.com/cwhelan/cloudbreak.
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Title: Combined effects of nicotine and ethanol on conditioned reward and neuroadaptation in DBA/2J Mice

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Nicotine (NIC) and ethanol (EtOH) share a high rate of co-abuse. We tested the hypothesis that NIC combined with EtOH has enhanced rewarding and neuroadaptive effects, using behavioral sensitization (a model of behavioral neuroadaptation) and conditioned place preference (CPP; a model of conditioned reward). We used DBA/2J mice, an inbred strain that develops EtOH-induced CPP and sensitization. For the sensitization study, mice were treated with saline, NIC, EtOH, or NIC + EtOH with activity assessed every third day. On the final day, all mice received an EtOH only challenge. NIC + EtOH (2 g/kg) resulted in greater sensitization during the acquisition period and a similar locomotor response to 2 g/kg EtOH alone on the challenge day, compared to the effect of EtOH alone during acquisition. NIC + 1 g/kg EtOH did not enhance locomotor sensitization during acquisition and mice repeatedly treated with NIC + 1 g/kg EtOH had a reduced response to the 1 g/kg EtOH challenge, compared to mice treated with EtOH alone during acquisition. This suggests that the combined effects of NIC + EtOH on sensitization are dependent on the dose of EtOH and on whether testing is performed after treatment with the drugs in combination or with EtOH alone. In the CPP experiment, we measured preference induced by NIC (1 or 2 mg/kg), EtOH (1 g/kg) or NIC + EtOH, using a standard CPP procedure. Neither dose of NIC alone produced CPP. EtOH alone and EtOH + 1 mg/kg NIC produced similar CPP. EtOH + 2 mg/kg NIC failed to produce CPP, indicating that the higher NIC dose interfered with conditioning. These results suggest that NIC does not enhance the conditioned rewarding effects of EtOH. [Department of Veterans Affairs, P60AA010760, R24AA020245, F31AA020732 and a grant from the APA.]
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Title: Involvement of the dorsal hippocampus in cocaine conditioned place preference

Leah N. Hitchcock, Oregon Health & Science University

A key aspect of substance abuse is that drug taking often occurs in a specific context. As a consequence, exposure to drug-associated contexts can trigger cravings and relapse, even after long periods of abstinence. Although many studies have demonstrated that the hippocampus is critical for developing and retrieving contextual and spatial memories, very little is known about the role of the hippocampus in the contextual control of drug seeking. We examined the effects of hippocampal inactivation on expression and extinction of cocaine-induced conditioned place preference (CPP) in mice. During acquisition of CPP, distinct tactile cues were paired with cocaine (20 mg/kg, intraperitoneal, CS+) and different tactile cues were paired with saline (CS-) on alternate days. Groups differed in whether the CS+ and CS- cues were presented in the same large space (one-compartment procedure) or distinct small spaces (two-compartment procedure). Acquisition of CPP was promoted by the two-compartment procedure. Extinction, when mice were exposed to the CS+ cues in the absence of cocaine, was promoted by the one-compartment procedure. These findings suggest that a two-compartment configuration facilitated acquisition and attenuated extinction of a cocaine-induced CPP. Inactivation of the dorsal hippocampus (DH) with a microinjection of the $GABA_A$ agonist, muscimol, decreased expression of CPP after acquisition and increase expression of CPP after extinction. These effects differed depending on the spatial configuration, suggesting that the hippocampus may differentially modulate drug seeking following acquisition and extinction of CPP.
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Title: Wavelength And pH Dependent Detection of Homocysteine

Aabha Barve, Mark Lowry, Robert M. Strongin, Portland State University

Elevated levels of homocysteine in human plasma have been associated with neural tube defects cardiovascular and Alzheimer diseases. Whereas, research has shown that the deficiency of cysteine is associated with conditions such as slow growth in children, hair depigmentation, liver damage, muscles and fat loss. Therefore, it is important to detect the physiological concentrations of cysteine and homocysteine in human plasma. In the past, many techniques such as spectrophotometry, fluorimetry and electrochemical methods have been used to detect these biomolecules, but despite being sensitive, cysteine and homocysteine responses interfere with each other. Previously, we have reported that the fluorescence quenching of fluorescein dialdehyde at pH(9.5) in response to cysteine and homocysteine due to the formation of 5 and 6 membered heterocycles respectively. Herein, we are trying to optimize the system by evaluating several pH values and excitation wavelengths. We show that the response of fluoresceine dialdehyde to thiols is dependent on pH, excitation wavelengths and time. In our future work, we aim to improve the detection limit of homocysteine and cysteine and understand the mechanism of reaction of these aldehyde bearing chromophores with amino thiols.
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Title: The Polyamine Pathway as a Potential Therapeutic Target against Leishmaniasis

Fernando Estrada[1*], Euna Kim[1], Tamara Olenyik[1], Estaban A. Panozzo-Zenere[2], Guillermo R. Labadie[2] and Sigrid C. Roberts[1**]
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Polyamines are organic cations found in almost every eukaryotic cell. These metabolites play an important role in cellular processes including growth, differentiation, and the biosynthesis of macromolecules; therefore they are extremely important for rapidly proliferating cells. Because of this significance, research has focused on the polyamine pathway as a potential therapeutic target against cancer and parasites. In fact, DL-α-difluoromethylornithine (DFMO), an irreversible inhibitor of the first enzyme in the polyamine pathway, has been proven successful in the treatment of late-stage African sleeping sickness, a disease caused by the parasite *Trypanosoma brucei gambiense*. Gene deletion mutants as well as overproducer strains have been previously generated in order to explore the polyamine pathway as a potential therapeutic target in *Leishmania* parasites. These studies demonstrated that the enzymes of the polyamine pathway are indeed important for parasite infectivity. The aims of the present study were: 1) To determine whether antileishmania agents exert their efficacy by inhibiting enzymes involved in the polyamine pathway, and 2) To determine whether arginine analogs shown to inhibit the recombinant enzyme arginase are effective against the *Leishmania* parasites. A small library consisting of 30 polyamine analogs were screened in *Leishmania* parasites, and in order to identify compounds that target the polyamine pathway supplementation with polyamines was utilized. The arginine analogs, N^{ω}-hydroxy-L-arginine (NOHA) and N^{ω}-hydroxy-nor-L-arginine (norNOHA), showed moderate efficacy against the *Leishmania* parasites which was completely alleviated by polyamine supplementation demonstrating that the enzyme arginase is being targeted in intact parasites. Potency of these analogs varied between the different *Leishmania* species indicating that the response to the therapeutic treatment varies with each *Leishmania* species. Our research reveals that the polyamine pathway can indeed be a potential therapeutic target against *Leishmania* parasites; however, a more high-throughput screening approach would be ideal for future studies in order to identify more potent compounds.
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Title: Development of a novel therapeutic strategy to alleviate doxorubicin induced cardiotoxicity though the use of polyphenols in nano-carrier formulation for chemotherapy

Brianna Cote (Presenter) and **Deepa A. Rao** (Mentor), Pacific University

Doxorubicin HCl (DOX), used to treat solid tumors, is highly effective but is limited by its cardiotoxic effects. Resveratrol (RES) and quercetin (QUE) are natural products that have demonstrated anticancer and antioxidant properties *in vitro* and *in vivo*. However, RES and QUE have low aqueous solubility and require a micellar formulation to achieve clinically relevant concentrations. We hypothesize that co-administration of RES and QUE in micelles with DOX may alleviate DOX induced oxidative stress while increasing/retaining its potency. The goal of our work is test this hypothesis *in vitro* in cardiomyocytes (H9C2) and in ovarian cancer (SKOV-3) cells.

RES and QUE 1:1 molar ratio micelles in Pluronic® F127 were prepared and characterized for size, loading, stability and *in vitro* drug release. Cytotoxicity experiments in H9C2 and SKOV-3 cells were performed with RES, QUE, DOX, RES:QUE:DOX (RCD) 10:10:1 and RES:QUE micelles: DOX (mRCD) 10:10:1 using Cell Viability Assay. Combination index (CI) analysis was used to determine synergism and antagonism in drug combinations in the cells.

RES:QUE micelles of 1 and 1.33 mg/mL with 25 nm diameter, were stable for 48 hr. RES:QUE micelles were able to release the drugs over 48 hr under sink conditions. In SKOV-3, the IC_{50} values for RES, QUE, and DOX were 97.36±0.85 µM, 56.64±0.55 µM, and 1.14±0.41 µM respectively. In H9C2 cells RES, QUE, and DOX IC_{50} values were 171.18±1.14 µM, 247.37±1.05 µM, and 6.38±0.26 µM respectively. CI index analysis indicates that RCD and mRCD were synergistic in SKOV-3 and antagonistic in H9C2 cells.

Based on the preliminary work, a potential chemotherapeutic strategy for alleviation of DOX induced cardiotoxicity while increasing its potency by concurrent treatment with RES:QUE 1:1 micelles has been proven feasible *in vitro.* Further studies *in vivo* are needed to determine the full therapeutic potential for this treatment strategy.

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APPRENTICESHIPS IN SCIENCE AND ENGINEERING (ASE) PROGRAM:

Saturday Academy High School Students, Summer 2013 Internships at universities noted:

Title: Risk for Methamphetamine Use Involves Sensitivity to Opiates
Katrina Matthews (Oregon Health & Science University internship)

Title: Isolation and Detection of Biomolecules in Licorice
Nguyen Truong (University of Portland internship)

Title: Genomic Study of the Extremophile Virus SSV1
Valerie Huang (Portland State University internship)

Title: Communication and Cooperation in Bacteria
Molly Unsworth (Oregon State University internship)

Title: Physicochemical Properties of Carbon Nanotubes that Relate to their Toxicity
Hattie Greydanus (Oregon State University internship)

Title: Extraction, Isolation, and Biological Activity of Chemicals in Betel Leaf
Vy Li (University of Portland internship)