

Lecture 12: Reporting progress and (unexpected) challenges

COSC 526: Introduction to Data Mining
Spring 2020



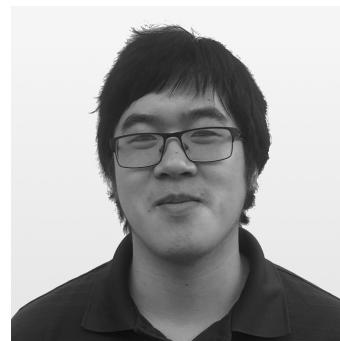
THE UNIVERSITY OF
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Instructor:



Michela Taufer

GRA:



Nigel Tan

Experts:



Leobardo Valera



Mike Wyatt

Project

Define your project (March 13)

- Which dataset will you be using? How are you obtaining the data? (We will provide you the appropriate data from NHANES or Medicaid, if you choose to use either of these datasets.)
- What is (are) the scientific question(s) that you want to answer? Be as specific as possible.
- What is your strategy to answer the question(s)? Define a set of steps that, if implemented with your code, will allow you to answer the question(s). Be as specific as possible.
- What is the tentative title of your project?

Create a new notebook with your solution (March 27)

- Write down the steps of your solution in distinct text cells; add one or multiple cells (as needed) to hold your code for each step. You can leave these software cells empty for the moment. Expand the text cells describing your solution.
- Add visualization cells that allow you to visualize results. You can leave these software cells empty for the moment.
- Add software to the code cells that upload data from source and pre-process data.
- Push your notebook into your GitHub repository as frequently as needed.

Finalize software and run tests within your notebook (April 3)

- Add the software that implements the method (or methods) to analyze your data.
- Add visualization cells that allow you to visualize results
- Push your notebook into your GitHub repository as frequently as needed.

Build a set of 15 slides that describe your work and get feedback (April 17)

- Build a set of ppt slides (use template provided) that summarize your work; use text slides to tell the story of your project and figures with the key results of your work.
- Make sure your slides include: motivation and problem definition, related work and background, your methodology (e.g., with flowcharts and code sections), your results, summary, and conclusions.

Create your poster and get feedback (April 24)

- Copy and paste your slides into the poster template.
- Shuffle as needed, extend and fill gaps, embellish fonts and text, enlarge text and figures to make them readable.
- **Submit your poster in GitHub (April 24)**
- **We will create a webpage with all your posters**

Poster layouts

Poster 1

- Poster from:

<http://betterposters.blogspot.com/2011/04/critique-breast-cancer-inhibition.html>

O⁶-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Resensitizes Breast Cancer Cells to Anti-Estrogen Therapy

Joshua Smith¹, George C Bobustuc¹, Rafael Madero-Visbal¹, Jimmie Colon¹, Beth Isley¹, Jonathan Ticku¹, Kalkunte S. Srivenugopal and Santhi Konduri^{1*}

¹Cancer Research Institute of M.D. Anderson Cancer Center Orlando ²Texas Tech University Health Sciences Center, Amarillo, TX

Abstract

Endocrine therapies using anti-estrogens are least toxic and very effective for breast cancers, however, tumor resistance to tamoxifen remains a stumbling block for successful therapy. Based on our recent study on the involvement of the DNA repair protein MGMT in tamoxifen resistance [Cancer Letters 15, 6087; 2009], here, we investigated whether MGMT overexpression mediates tamoxifen resistance. We found that tamoxifen resistance was significantly reduced in tamoxifen-treated (BG) cells at a non-toxic dose alone or in combination with the anti-estrogens (tamoxifen/favostatin) curtails human tamoxifen resistant breast cancer cell growth. Further, we also determined whether BG sensitizes breast cancers to tamoxifen using tamoxifen resistant cells.

Mgmt expression was found to be increased in breast cancer cells relative to normal breast epithelial cells. Also, Mgmt levels were significantly higher in tamoxifen resistant cells than in tamoxifen sensitive cells. A series of DNA-damaging alkylating agents attack the O⁶-methylguanine (O⁶-MG) on guanine-containing nucleotides and highly cytotoxic damaged DNA crosslinks. The DNA repair enzyme O⁶-alkylguanine DNA alkyltransferase (AGT), encoded by the gene MGMT, repairs alkylation at this site and is responsible for protecting both tumor and normal cells from alkylating agents. Mgmt is expressed in most breast cancer cells. In tamoxifen resistant cells, the expression of tamoxifen accelerated proteasomal degradation of Mgmt in human cancer cells. In 1991, Pegg, Moesch, and Dolan observed that O⁶-benzylguanine (BG) inhibited AGT and potentiated the cytotoxicity of both epoxymethylation and methanesulfonate. In a series of important studies, Pegg et al. demonstrated that BG is a potent AGT inhibitor. BG is not incorporated into DNA in living cells and reacts directly with the active site of AGT. BG inhibits AGT in MCF-7 cells. BG inhibits AGT in MCF-7 cells by preventing transfer of heavy group at the active site of AGT. The Mgmt protein is degraded after each reaction. This stoichiometric reaction mechanism effectively depletes the AGT content in tumors and the associated repair of alkylation damage. BG is currently undergoing clinical trials in various cancers to increase the efficacy of alkylating agents.

Introduction

Recent advances in breast cancer research have key pathways involved in the repair of DNA damage induced by chemotherapeutic agents. The ability of cancer cells to recognize DNA damage and initiate DNA repair is an important mechanism for therapeutic intervention and has a major impact on cancer outcome. A series of DNA-damaging alkylating agents attack the O⁶-methylguanine (O⁶-MG) on guanine-containing nucleotides and highly cytotoxic damaged DNA crosslinks. The DNA repair enzyme O⁶-alkylguanine DNA alkyltransferase (AGT), encoded by the gene MGMT, repairs alkylation at this site and is responsible for protecting both tumor and normal cells from alkylating agents. Mgmt is expressed in most breast cancer cells. In tamoxifen resistant cells, the expression of tamoxifen accelerated proteasomal degradation of Mgmt in human cancer cells. In 1991, Pegg, Moesch, and Dolan observed that O⁶-benzylguanine (BG) inhibited AGT and potentiated the cytotoxicity of both epoxymethylation and methanesulfonate. In a series of important studies, Pegg et al. demonstrated that BG is a potent AGT inhibitor. BG is not incorporated into DNA in living cells and reacts directly with the active site of AGT. BG inhibits AGT in MCF-7 cells. BG inhibits AGT in MCF-7 cells by preventing transfer of heavy group at the active site of AGT. The Mgmt protein is degraded after each reaction. This stoichiometric reaction mechanism effectively depletes the AGT content in tumors and the associated repair of alkylation damage. BG is currently undergoing clinical trials in various cancers to increase the efficacy of alkylating agents.

Interestingly, several observations suggest an inverse correlation between the levels of MGMT and p53 tumor suppressor protein where wild-type p53 suppresses transcription of MGMT mRNA. In contrast, if p53 expression is often inactive or suppressed in human cancers, its induction or restoration of p53 function is an important goal of cancer treatments. However, when this is not resolved by suppression of MGMT expression has yet to be determined. Thus, the cross-treatment of MGMT and ERα (and the link to p53 expression) has not been explored in drug (i.e., tamoxifen)-resistant breast tumors. The anti-estrogen tamoxifen is the most commonly used treatment for patients with estrogen receptor positive breast cancer. In this setting, the potential for resistance to tamoxifen is a significant problem. The primary goal of present study was to investigate the mechanisms of anti-estrogen drug resistance and to design new therapeutic strategies for circumventing this resistance. The results show that MGMT expression is an important clinical problem. The primary goal of present study was to investigate the mechanisms of anti-estrogen drug resistance and to design new therapeutic strategies for circumventing this resistance. The results show that MGMT expression is increased in TAM-resistant breast cancers and inhibition of MGMT by BG significantly improves TAM-sensitivity.

Results

Prolonged Treatment of Tamoxifen Increases MGMT Expression: We developed a tamoxifen resistant MCF-7 cell line using prolonged treatment of tamoxifen on the parental ER⁺/breast cancer cell line, MCF-7. Tamoxifen-resistant MCF-7 cells proliferate at rates similar to the parental MCF-7. Prolonged treatment of tamoxifen onto MCF-7 cells increased MGMT expression compared to parental MCF-7 cells (Fig.1).

Knocking Down ERα Enhances MGMT Expression in Tamoxifen Resistant Breast Cancer Cells: It is not known whether ERα is involved in MGMT expression. We also addressed whether ERα is functionally required after tamoxifen treatment breast cancer cells. Therefore we investigated whether down regulation of ERα has any effect on endogenous MGMT expression in these cells. As expected, downregulation of ERα using specific siRNA significantly reduced ERα protein levels in these cells. Western blot analysis was performed and the results in Fig. 2A show that ERα protein levels were significantly reduced in tamoxifen treated cells. The level of MGMT expression in these cells, and interestingly, the results in the right panel (Fig.2B) show increase MGMT mRNA levels were increased as assessed by qRT-PCR. These data suggest that ERα-signaling functions to regulate MGMT gene expression in breast cancer cells.

Transcriptional Regulation Between MGMT and p53: Previously, it was reported that p53 negatively regulates MGMT in breast cancer cells. Therefore, we addressed whether or not silencing the p53 enhances endogenous MGMT expression. Tamoxifen resistant MCF-7 cells were transfected with either p53 siRNA (p53-KD) or Non-specific siRNA (NS). MGMT expression was measured by qRT-PCR. The results clearly demonstrate that BG significantly enhanced p53 transcriptional activity by 4-fold in these cells (Fig.4).

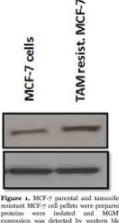


Figure 1. MCF-7 parental and tamoxifen-resistant cells were prepared. proteins were isolated and total cellular protein was analyzed by Western blot analysis. Tamoxifen-resistant MCF-7 breast cancer cells significantly increased MGMT expression compared to MCF-7 parental cells.

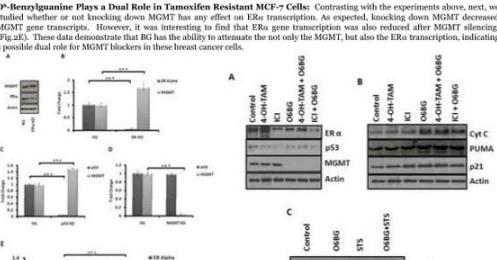


Figure 2. (A) Tamoxifen resistant MCF-7 breast cancer cells were treated with increasing doses of BG (0 ng/ml and 200 nM) for 48 h. Total ERα and MGMT protein was determined by western blot analysis. MGMT protein was significantly increased with 200 nM BG. (B) PEG-PCR analysis of tamoxifen resistant MCF-7 cells treated with BG and control. (C) MGMT mRNA was determined by qRT-PCR. MGMT transcription was significantly increased in ERα knockdown cells. (D) MGMT mRNA was determined by qRT-PCR. BG significantly increased MGMT mRNA in tamoxifen resistant cells. (E) BG (200 nM) knock down tamoxifen resistant MCF-7 breast cancer cells were treated with BG (200 nM) for 6 hrs. PARP cleavage was determined by western blot analysis.

O⁶-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Increases Tamoxifen Resistant Breast Cancer Cell Sensitivity to Anti-Estrogen Therapy: TAM/ICL stained micrographs revealed that all the mice had tumors in the breast. The data summarized in Table 1 shows the daily BG alone or in combination with twice weekly tamoxifen/ICI significantly decreased median tumor volume and weight as compared with that seen in tamoxifen/ICI treated and control mice. The combination of BG with tamoxifen or ICI produced the best response in terms of tumor volume and weight reduction (Table 1). Tumor weight was also significantly reduced in mice treated with combination therapy as compared with control mice (81.23 mg, 22.39 mg (TAM+BG), respectively, p<0.0001; 81.23 mg, 51.57 mg (ICI+BG), respectively, p<0.0005) (Table 1). Body weight was not significantly different in all the treatment groups. All the mice had visible liver metastases were present (enumerated with the aid of a dissecting microscope) in all treatment groups.

Histology & Immunohistochemistry: We next determined the in vivo effects of BG (alone or in combination) with tamoxifen/ICI. Tumors harvested from different treatment groups were processed for routine histological and IHC analysis. Tumors from mice treated with BG alone or in combination with tamoxifen/ICI exhibited a significant decrease in MGMT, ERα, ki-67 as compared with tumors treated with tamoxifen/ICI alone or control group. p53 expression was significantly increased in tumors from mice treated with BG alone or in combination with tamoxifen/ICI. The expression of p53 was significantly increased in tumors from mice treated with BG either alone or in combination with tamoxifen/ICI. The images were analyzed by ImageJ (NIH) and MGMT, ERα, p53, p21 and ki-67 expressions were quantified by the ImmunoRatio plugin (Fig.5).

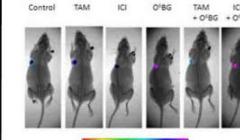
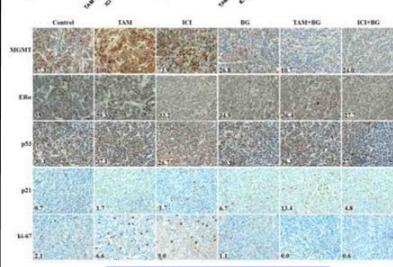


Figure 3. (A) Tumors from mice bearing MCF-7 breast cancer cells were treated with increasing doses of BG (0 ng/ml and 200 nM) and tamoxifen (TAM, 200 nM) either alone or in combination with BG. 200 ng/ml tamoxifen treated cells were harvested and isolated and both subcutaneously transplanted into nude mice. (B) PEG-PCR analysis of tamoxifen resistant MCF-7 cells treated with BG and control. (C) MGMT mRNA was determined by qRT-PCR. MGMT transcription was significantly increased in ERα knockdown cells. (D) MGMT mRNA was determined by qRT-PCR. BG (200 nM) knock down tamoxifen resistant MCF-7 breast cancer cells were treated with BG (200 nM) for 6 hrs. PARP cleavage was determined by western blot analysis.

Figure 4. Tumors were harvested from control mice and mice treated with tamoxifen/ICI. The sections were immunostained for expression of MGMT, ERα, p53, and ki-67. The expression of MGMT, ERα, p53 and ki-67 was significantly decreased in tumors from mice treated with tamoxifen/ICI. BG either alone or in combination with tamoxifen/ICI significantly decreased MGMT, ERα and ki-67. p53 expression was not much affected in tumors from mice treated with tamoxifen/ICI. In contrast, expression of p21 was significantly increased in all these treatment groups compared to control. Representative sample (x40) are shown.



Conclusions

- In the present study, we observed that prolonged treatment with anti-estrogens causes drug resistance by inducing the DNA repair protein O⁶-methylguanine DNA methyltransferase (MGMT).
- Decreasing the expression of MGMT by exposing breast cancer cells to BG sensitized these cells to anti-estrogen therapy (TAM/ICI).
- We also observed that combination therapy of anti-estrogens and MGMT blockers not only overcome the MGMT derived drug (tamoxifen and ICI) resistance but also increased the efficacy of anti-estrogen therapy by decreasing estrogen receptor expression and restoration of the functional activity of p53 in tamoxifen resistant breast cancer cells.
- Combination therapy inhibited tamoxifen resistant breast tumor growth *in vivo*.

Acknowledgements

We would like to thank the Florida Department of Health, Sunshine State Cancer Program (S3) for their funding of the project.

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More about how to prepare posters

- The following set of slides are from this extended lecture:

https://urc.ucdavis.edu/sites/g/files/dgvnsk3561/files/local_resources/documents/pdf_documents/How_To_Make_an_Effective_Poster2.pdf



How To Make An Effective Poster



Undergraduate
Research
Center

Matthew Stuckey, PhD(c), MPH(c)
Program Coordinator

Tammy Hoyer
Assistant Director

With information kindly provided by Lolita Adkins and Jeremy Foin



What is an Academic Poster?

- A form of Academic Expression
- Summary of Research (5 – 10 minutes)
- Visually augmented discussion/interaction
- At conferences viewers come to you (or you can invite)
 - People search published abstracts
 - Posters may be grouped by field & folks may wander
- New Information
- Characteristic Fields
- Appearance/Content varies by Field or Lab



Why are Academic Posters Important?

- Represents you and your sponsor's research at:
 - Conferences
 - Symposia
 - Hallways
 - Informational Days
- Demonstrate expertise
- Demonstrate attention to detail
- Practice public speaking
- Learn about most current results in field
- Deepens understanding of topic
- Opportunity for teaching and learning
- Share ideas
- Create collaborations



Your Audience will be??

- Researchers in your field will read even if bad
- Researchers in related fields easily persuaded to view
- Previously uninterested passers by can be attracted by a good poster
- ***You want to attract these people!***
- Don't vary content, vary explanation



Main Elements of a Poster

- Title (same as submitted abstract)
- Name and Campus
- Core Technical Content
 - Abstract
 - Introduction
 - Results
 - Discussion
 - Literature cites/Resources
 - Acknowledgements
- Visuals
- Font should be legible fonts like:
 - Times New Roman
 - Arial
 - Garamond
 - Berkeley UC Davis Medium
- Do not use illegible fonts like:
 - *Brush Script*
- Use the same font type throughout your poster
- No smaller than 16 pt. font



types of contrast

size | 

texture | 

position | 

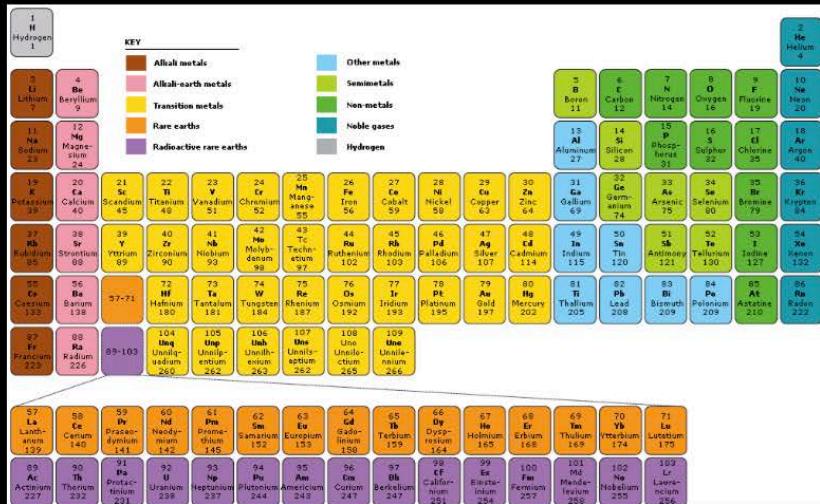
shape | 

color | 

orientation | 

color

- color theory is an extremely complicated topic that could take up an entire class on its own
- for our purposes we will focus on two aspects:
 - color as an emotional tool
 - color as an organizational tool



color temperature – warm or cool?



color temperature - warm or cool?



color temperature

warm vs. cool colors

- warm
 - hues from red through yellow, including browns and tans
 - seem to advance or appear more active; often evoke feelings of happiness, optimism and energy, but can be visually overwhelming
- cool
 - cool = blue-green through blue-violet, including most grays
 - appear to recede into the background; usually calming and soothing, but can also express sadness

color as an organizational tool



Purpose:
To study iron protein biochemistry from the perspective of the iron
Protein = Ligand

TRANSFERRIN
A mechanistic study of the iron release by receptor-bound transferrin using spectroelectrochemistry

FERRIC BINDING PROTEIN
Role of a synergistic anion on modulating iron uptake in a bacterial transferrin by pathogenic bacteria: A study in kinetics and thermodynamics

HEMOGLOBIN
Effects of subunit cross-linking on hemoglobin oxidation states determined by spectroelectrochemistry

Duke University – Department of Chemistry – Durham, NC

The Iron Paradox
Iron is needed for nearly every living cell
Iron is toxic and can produce reactive oxygen species & must be controlled

Iron Abundance In Humans
45-55 mg/kg in humans
70% in Red Blood Cells (hemoglobin)
8.5% in Transferrin
However:
Turnover of transferrin iron is ~30 mg / 24 hours with 80% of this Fe being transported to the bone marrow for hemoglobin synthesis
Bacteria can also target Tf as a source of iron

Techniques:
Spectroelectrochemistry
UV-Visible Spectroscopy
Fluorescence Spectroscopy
Difference Spectroscopy
Stopped-Flow Kinetics
SUPREX

Transferrin
Spectroelectrochemistry utilizes a short pathway created by an OTTLE cell, to measure the variations in visible spectra as the analyte is oxidized or reduced by an externally applied potential. This technique is ideal for a biological analyte because only a small sample volume is required.

Heterogeneous reactions are complicated because the reaction can be limited by the product and/or the reagent physically close to the electrode surface. Mediators are used to act as electron shuttles

Iron located Tf binds to the human receptor and is released into the blood by endopeptidase. Tf releases iron in the plasma or in the extracellular fluid. The buffers are acidic (Andrews, 1999). However, the chemical mechanism is unclear. The reduction potential of Fe³⁺ in the plasma (pH 7.4) and in the extracellular fluid (pH 6.8) is too low for biological reducing agents.

Reduction potential of Fe³⁺ upon receptor binding

The transferrin receptor is capable of shifting the reduction potential into the range accessible by biological reducing agents, allowing for a redox mechanism of Fe release. Transferrin not only supplies iron to mammalian cells, but has been identified as a target for pathogens to metabolically steal iron from their host

How is Fe³⁺ removed from Tf when $K_m = 10^{-2} \mu M$?

Hypothesis: When transferrin binds to a receptor, the reduction potential shifts into a biologically relevant range.

Pyridine nucleotide reduction of Tf

Tf can be scavenged by receptors on the bacterial surface. FbpA is a nodal point in this iron acquisition process.

Both proteins utilize an exogenous anion

Structural Fe binding site similarity between Tf and FbpA

Reduction potential values by ~140 mV (14 kJ) based on identity of X.

Iron transport can occur by a redox or non-redox mechanism in the plasma. The thermodynamic stability and reduction potential are governed by the identity of the synergistic anion. Kinetically labile exchange is possible in the diverse anionic conditions of the plasma.

Line 1: FbpA requires a synergistic anion to facilitate tight IgM binding, which may play a role in ease and rate of Fe uptake by the bacterium.

1. FbpA acts as an iron binding protein

2. FeFbpA-X can exchange anions

3. Anion identity modulates both thermodynamic stability and redox potential

Fe³⁺ + FbpA-X → Fe²⁺FbpA-X

Thermodynamic stability varies by two orders of magnitude (14 kJ) based on identity of X.

Iron transport can occur by a redox or non-redox mechanism in the plasma. The thermodynamic stability and reduction potential are governed by the identity of the synergistic anion. Kinetically labile exchange is possible in the diverse anionic conditions of the plasma.

Chemically modified Hb

- Pyridylation
- Pegylation
- Conjugation to polysaccharides & proteins

HbA₂

-Intramolecular cross-linking

Combined Nernst Plot – Combined Hill Plot

E_{1/2} vs Log p_{O₂}

Implications

- Reoxygenating redox center not necessary
- Drive for cooperativity
- Structural perturbations
- Structural modifications perturb kinetics by altering exposure of heme cavity

Modified Hb Conclusions

Sample	E _{1/2} mV (NH ₄) ₂ S	Oxidation	Log p _{O₂}	Oxygenation
HbA ₂	83	1.2	-0.455	2.28
Hemoglobin	97	0.7	0.994	0.71
Des-BTC	94	0.9	0.818	1.40
OxyD ₂ BTC	106	0.8	1.028	1.11
aa-DBBP	125	1.0	0.461	1.58

Anaerobic Reduction Potentials

- Loss of cooperativity
- E_{1/2} potential increased vs HbA₂
- Normal physiological range
- Decreased tendency to form methb

Keller, Zik, Aszen, and Crimmins. (1998) *Inorg. Chem.* 37, 3664

Dhungana, Tobby, Anderson, Vaughan, Aszen, Meierhofer, and Crimmins. (2003) *PNAS* 100, 3639-64

Dhungana, Tobby, Zik, Levine, Crimmins, and Aszen. (2004) *Biochem.* 43, 205-6

Heymann, Weissen, Matzner, and Crimmins. (2000) unpublished

Dhungana, Anderson, Matzner, and Crimmins. (2000) *Biochem.* 44, 3905-18

Rouffell, Powell, Dhungana, Weissen, Matzner, Crimmins, and Flanagan. (2004) *Biochem.* 43, 15737-74

Dhungana, Tobby, Anderson, Vaughan, Aszen, Matzner, and Crimmins. (2003) *PNAS* 100, 3639-64

Bonaventure, Henkens, Weissen, Henrich, Pearce, Alayash, and Crimmins. (2006) unpublished

Tobby, Bonaventure, and Crimmins. (2002) *Meth. in Enzymology* 352, 187-209

Reiss (2001) *Chem. Rev.* 101, 2797-2919

proximity

- moving elements closer or farther apart to achieve a more organized look
- based on the idea that related items in close proximity will be perceived as a unified group
- your audience will respond by:
 - a) tending to naturally group similar items that are near to each other into a single unit, and
 - b) assuming that items that are not near each other in a design are not closely related to one another

alignment

- arranging elements so that they line up
 - creates order
 - organizes page elements; links disparate groups into a unified whole
 - satisfies the subconscious human desire to line things up (I'm not kidding, this is an actual thing)
 - creates imaginary visual connections

**ignore alignment at
your own peril!**

this poster has some serious alignment issues...

Salvage Archaeology at the Snake River Sandspit Site in Nome, Alaska

Concurrence of No Historic Properties:

• March 19, 1998 – The Corps sent a letter to the SHPO requesting concurrence that their project to improve the harbor at Nome, Alaska "does not have the potential to affect cultural resources."

• April 29, 1998 – The Corps received a letter from the SHPO, in which she concurred that "there are no historic properties in the area of potential effect."

Despite this, the Corps thought it was a good idea to have an archaeological monitor on site during the groundbreaking. A private archaeologist familiar with the area was subcontracted to monitor the initial construction during May 2005.



First evidence of the second house pit (Locality B), discovered by Corps archaeologist Megan Grover and bulldozer operator Mike Ulrich.

Discovery of the Site (Locality A):

• 1st week of May, 2005 – The subcontracted archaeologist identified the remains of a semi-subterranean house pit while monitoring the construction.
• The archaeologist took photographs and recovered approximately 25 artifacts, then decided that the house pit was ineligible for inclusion on the National Register of Historic Places and allowed the bulldozers to push the remains into the ocean.

• May 14, 2005 – The Corps received a letter from the subcontracted archaeologist mentioning the discovery and subsequent destruction of the semi-subterranean house pit.
• May 26, 2005 – The Corps sent a letter to the SHPO stating that the house pit is "not eligible for the National Register for Historic Places" because it "has lost integrity of design, materials, workmanship, and association."
• September 27, 2005 – The Corps sent a letter to Nome Eskimo Community (NEC), apologizing for not considering after the discovery of the site and stating that they will continue to work with the tribe to mitigate the damage done.

• October 28, 2005 – The SHPO sent a letter to the Corps in which she concurred with the "finding that the house pit no longer retains sufficient integrity to be eligible" and agreed that "appropriate mitigation could include the development of interpretive signs that discuss the Native history of the Nome area."

Nome Eskimo Community tribal Elder Al Zahn and Corps archaeologist Helen Linsenthal, excavating house pit B while construction of the revetment rock continues nearby.



Continued Discovery of the Site (Locality B and C):

• July 2006 – The Corps sent one of its own archaeologists, Megan Grover, to monitor the continued project construction.
• July 26, 2006 – Megan identified the remains of a second semi-subterranean house pit. She called the SHPO and left a telephone message about the discovery of the house pit, along with her contact information. She also contacted the City of Nome, Nome Eskimo Community (tribe), and Bering Straits Native Corporation. She called the SHPO again and spoke with a Review and Compliance Archaeologist at the SHPO's office, who agreed that she should excavate a test pit and do some shovel screening to identify the boundaries of the feature.

• July 27, 2006 – Megan called the SHPO again and left another telephone message about the site.
• July 28, 2006 – Megan called the SHPO again and talked with a Review and Compliance Archaeologist at the SHPO's office. Megan told the SHPO archaeologist that she was assuming the site was eligible for the National Register, and that she was going to excavate at least 50% of the site.

• August 3, 2006 – A meeting was held in Nome between the Corps, the Nome Eskimo Community, and the City of Nome, with the SHPO participating via teleconference, to discuss the discovery of the site and what to do about it.



Proposed Mitigation (as agreed upon in the draft MOA):

- 1) Write a site report (Data Recovery Report)
- 2) Provide for an accredited museum conservator to visit the City's Carrie M. McLain Memorial Museum and assist in the conservation and curation of the site artifacts on display
- 3) Assist with the accessioning of site artifacts and archaeofauna (bagging, cataloging, and appropriate photography)
- 4) Provide a museum-quality display case to the City's Carrie M. McLain Memorial Museum
- 5) Present information learned from the site in a series of public lectures in Nome
- 6) Prepare a manuscript on information learned from the site that can be utilized by Nome teachers (grades 5-12)
- 7) Present information learned from the site to a conference of peers
- 8) Submit an article about the site for publication in a peer-reviewed journal (if not accepted, publish elsewhere)

Excavating house pit B while heavy machinery runs nearby. Nome Eskimo Community employee Karlin Itchook and City of Nome employee Meghan Tim Elyck.



Discovery of the hunter's cache at the middle. Nome Eskimo Community employee Karlin Itchook, Corps archaeologist Arianne Wilson, and others.



Excavating the middle. Corps employees Mack Cassell, Ory McConnell, Megan Grover, Nome Eskimo Community tribal Elder Al Zahn, Kawerak employees.

The Excavation:

- Occurred from July 26, 2006 to August 26, 2006.
- Involved over 25 community volunteers, including:
 - City of Nome employees
 - Nome Eskimo Community (tribe) employees, members, and tribal Elders
 - Mr. Karlin Itchook, the tribe's Historic Preservation Representative, participated in the excavation every day
 - Kawerak, Inc. (regional non-profit Native corporation) employees
 - Interested Nome citizens
- Involved 6 Corps employees, including biologists and chemists as well as archaeologists and archaeology interns



Excavating house pit B. Nome Eskimo Community members Roger Johnson, Karlin Itchook, and Al Zahn, Corps archaeologists Helen Linsenthal and Mack Cassell, City of Nome employee Meghan Tim Elyck.



Corps archaeologist Megan Grover and King Island Native Community tribal Elders at a public viewing of site artifacts.



Public Outreach in Nome:

- Public viewing at Old St. Joe's Cathedral (August 10, 2006).
 - Over 200 people attended
- Viewing of artifacts at Nome Eskimo Community's building, for tribal members (August 2006)
- Viewing of artifacts at Kawerak's building during the regional shareholders meeting (August 2006).
- Another public viewing event at Old St. Joe's Cathedral (September 16, 2006).
 - Over 150 people attended
- Megan Grover gave a public lecture at the National Park Service's building (November 2006)



Corps archaeologist Megan Grover, Nome Eskimo Community tribal Elders Al and Margaret Zahn, and King Island Native Community tribal Elders of a public viewing of site artifacts.



Where We Are Today:

- Multiple drafts of the MOA have been sent out to signatories and consulting parties (on the following dates):
 - November 22, 2006
 - September 22, 2008
 - April 13, 2009
 - August 10, 2009
 - December 14, 2009
- After a statement meeting among the signatories to the MOA on December 15, 2009, and numerous unproductive meetings afterwards, advice was informally requested from the Advisory Council on Historic Preservation. On March 19, 2010, the ACHP sent the Corps an edited draft of the MOA.
- A new draft of the MOA is currently under discussion.
- Artifact and faunal analyses are being undertaken by Corps archaeologist Kelly Eldridge, and the Data Recovery Report is being drafted.

letter size

Q: how large should you make your type?

A: ***AS! LARGE! AS! POSSIBLE!*** THIS CANNOT BE OVEREMPHASIZED. MAKE IT AS BIG AS YOU CAN, THEN ADD ANOTHER 10% FOR GOOD MEASURE.

- *rule of thumb:* the smallest text on your poster should be clearly legible from 6 to 10 feet away
 - *at a minimum, type should be approximately:*
 - **72 points for titles**
 - **48 points for headings**
 - **24 points for body copy**

REMEMBER – THESE ARE MINIMUM VALUES!
BIGGER IS ALMOST ALWAYS BETTER
(within reason, of course)

Poster Overview- 36" by 48"

Sponsoring logo



Title: Should be seen from 4-5 feet away. Times New Roman or Arial, Bold, at 60-80 point text



**Title Line 1
Title Line 2**

Name Line (First, MI, Last)
Department of ?
University of California, Davis, 95616

Name: in 44 pts., bold
Department: 40 pts., bold
Institution: 40pts., bold



Institution Logo

INSERT ABSTRACT

Abstract: No more than 250 words

INSERT TEXT

INTRODUCTION

Heading: Legible font, bold, 44pts.
Section: Legible font, bold, 36 pts

**INSERT
FIGURE**

Figure 1: 32 pts, bold

INSERT TEXT

RESULTS

Heading: Legible font, bold, 44pts.
Section: Legible font, bold, 36 pts

INSERT TEXT

METHOD

Heading: Legible font, bold, 44pts.
Section: Legible font, bold, 36 pts

**INSERT
FIGURE**

Figure 2: 32 pts, bold

INSERT TEXT

DISCUSSION

Heading: Legible font, bold, 44pts.
Section: Legible font, bold, 36 pts

ACKNOWLEDGEMENTS
Legible font, 36 pts., bold

REFERENCES
Legible font, 36 pts., bold

First Thing First: The Title and Abstract

- The title of your abstract is very important
 - Reflect the content of the paper
 - Specific and Succinct
 - Use key words for indexing and for searches
- **250 Word Max**
- Includes the following:
 - The research question or problem
 - The methods
 - The observations
 - Analysis, assessment and implications
 - Major findings, results and conclusions
 - REVIEW WITH MENTOR

Abstract Example:

ANALYZING THE PHYSICAL INTERACTION BETWEEN Pch2 AND Cdc23 IN SACCHAROMYCES CEREVISIAE.

SOLIS, Ryan D., Senior, Neurobiology, Physiology, and Behavior Major, Dr. Sean M. Burgess, Department of Molecular Cellular Biology, University of California, Davis.

In sexually reproducing organisms, meiosis serves as a specialized form of cellular division that creates four haploid gametes from a single diploid cell. In prophase I of meiosis, homologous chromosomes physically interact by pairing and exchanging genetic material through recombination. This is followed by the separation of chromosomes during the first meiotic division. Inappropriate pairing and failed segregation of chromosomes can lead to improper chromosome rearrangements and aneuploidy. Furthermore, these errors can lead to birth defects, cancer and other diseases. In budding yeast, Pch2 protein is involved in a meiotic recombination checkpoint that is responsible for the proper segregation of chromosomes by arresting cells that show abnormal crossover patterns. To further investigate Pch2 functions, a yeast two-hybrid assay was used that tests for physical binding between Pch2 and potential interactors. The sequences isolated from positive interactors were compared to the yeast genome to search for homology between known proteins. Sequence homology search provided several possible protein interactors and from these results we have focused on conducting further studies with Cdc23. Cdc23 is an essential protein and part of a protein complex called the Anaphase Promoting Complex. This complex is known to participate in ubiquitination of targeted proteins involved in the progression through mitosis and the G1 phase of the cell cycle. Along with Pch2, we suspect that the APC may have a role in chromosome-protein structure. Currently we hope to use a GFP tag to view Cdc23 localization in the cell and create a meiotic null of the protein to further conduct studies to better understand its interaction with Pch2 during meiosis.

Title Example:

Does Perinatal Exposure to DDTs and the Development of Glucose Intolerance Promote Skeletal Muscle Deficiency?



Ciara Main₁, Michele La Merrill Ph.D₂
Department of Animal Science₁, Department of Environmental Toxicology₂, University of California, Davis



Introduction

- Or Background
 - This is separate from your abstract!
 - State the research question and significance of the study
 - Include related current investigations
 - If you are there, they won't read it so SAY IT!
 - Get viewers interested
 - Reason you chose to study
 - Foundation for your work (Models)
 - General topics to specific
 - Equivalent to 1 double spaced 12 pt page
 - Usually contain citations/references (cite!)
 - May have Purpose and Hypothesis embedded
 - Generally completes first column

INTRODUCTION

Various implant surface modifications, such as the application of hydroxyapatite (HA) coatings, have been reported to aid in accelerating osseointegration. These improvements in dental implant surfaces have allowed clinicians to replace missing dentitions more effectively and successfully in both fully and partially edentulous subjects. However, failures leading to implant removal still occasionally occur, and these failure occur either early following the installation of the implant or later when the implant supported reconstruction has been in function for various periods of time. In many instances, bacterial adhesion on implant surfaces has a strong influence on healing and long-term outcome of dental implants. In order to improve the life and success of implant therapy, there is a need to investigate the additive anti-bacterial effect in conjunction with the enhancement of rapid bone formation. Since the antimicrobial properties of the silver (Ag) have been exploited for a long time in the biomedical field, the objective of this study was to evaluate the initial anti-bacterial adhesion and osteoblast cell proliferation and differentiation on Ag-doped HA coating surfaces.

Introduction

- *Francisella tularensis* is highly infectious bacterium that causes the disease tularemia. *F. tularensis* has been classified as a potential biological weapon. There is currently no vaccine approved for human use, and its mechanisms of pathogenesis are poorly understood, in part because of a lack of genetic tools to study this organism.

- *F. tularensis* is divided into several subspecies, including the highly virulent (for humans) subsp. *tularensis*, the moderately virulent subsp. *novae-angliae*, and the low virulence (for humans) subsp. *powelli*.

- A cluster of genes, the *Francisella* Pathogenicity Island (FPI), has been shown to be essential for *F. tularensis* virulence.

- The FPI is duplicated in subspecies *holarctica* and *tularensis*.
- The IgC gene, located in the FPI, is essential for intramacrophagial growth and virulence in mice.

- A lack of efficient genetic tools have hampered the study of subspecies *holarctica* and *tularensis*. Moreover, the duplication of FPI genes has made the study of these genes in the more virulent subspecies cumbersome.

- We have developed a system for gene disruption in *F. tularensis* that utilizes a retargeted Group II Intron.

- This "Targetron" system works at high efficiency in subspecies *tularensis*, *holarctica*, and *nivalis*, and generates unmarked disruptions

INTRODUCTION

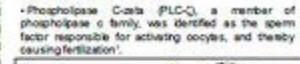


Figure 1. Eicosanoid action of PLC ϵ . (A) Measured rate of PI β_1 by PLC ϵ release from apical membrane. DAG = diacylglycerol; IP $_3$ = inositol triphosphate. (B) In *oocytes*, PI β_1 activates Ca $^{2+}$ channel of ER to release calcium. This hypothesis to produce Ca $^{2+}$ oscillation and spasmolytic fertilization.⁹

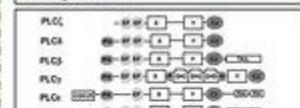
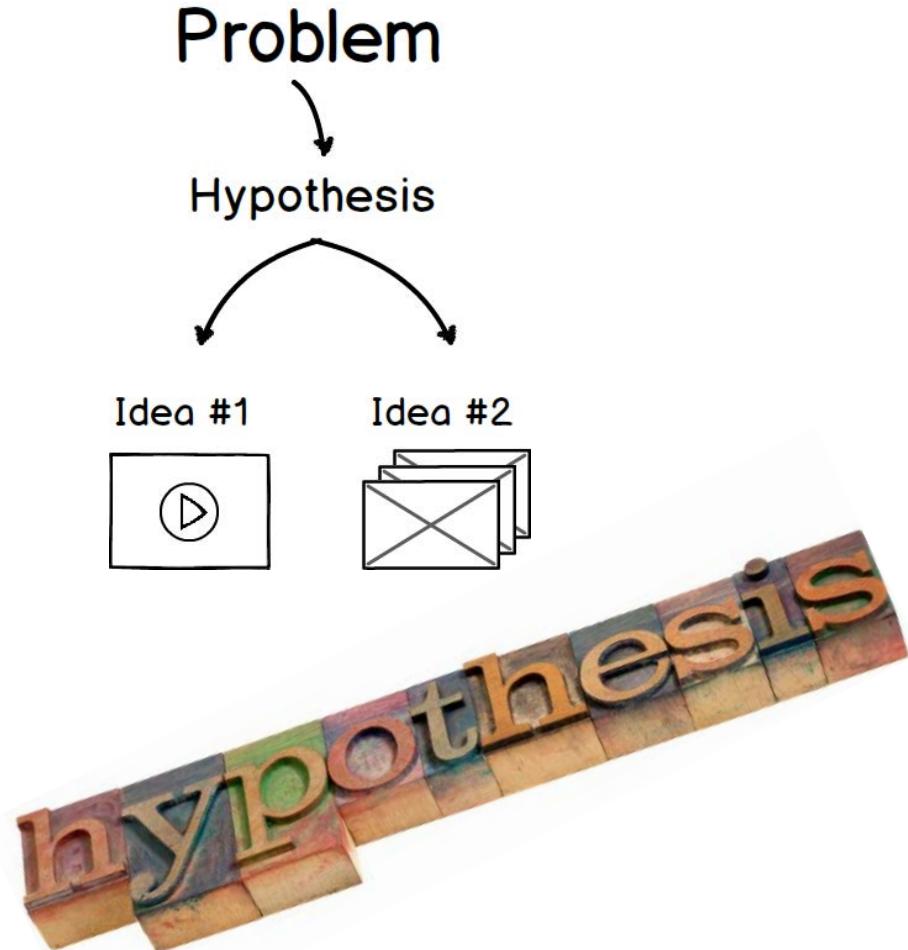


Figure 2. PLC Domain Organization. PLC- ζ consists of EF-hand domain, catalytic (X and Y) domain and C2 domain. These domains are also found in other PLC isoforms. PLC- δ showed closest resemblance to PLC- ζ .¹⁴

Bioinformatic analysis through sequence alignment and homology modelling revealed that the calcium binding region of C2 domain as well as the catalytic Y-region of PLC- ζ were expected to be significantly different from empirically determined PLC- β .

Purpose and Hypothesis

- Can be embedded in Introduction, but
- Sometimes a separate section, to emphasize
- Purpose or Objective, Aim, Goal, etc.,
- Why you did experiment?
- “The purpose of this project...”
- Good for Student Conference
- (Promotes solid judging)
- Hypothesis
- Same as for abstract



Methods

- Describe procedures and methods in detail to allow observer to understand how, when, where data was obtained.
- Describe challenges and lessons learned
- Text with subheadings
- Can include a flow chart to summarize
- May include citations
- Make sure to include:
 - subjects
 - experimental design
 - drugs and equipment used
 - statistical methods
 - why you chose the method

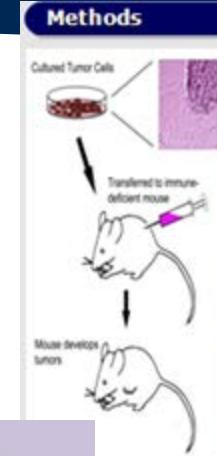
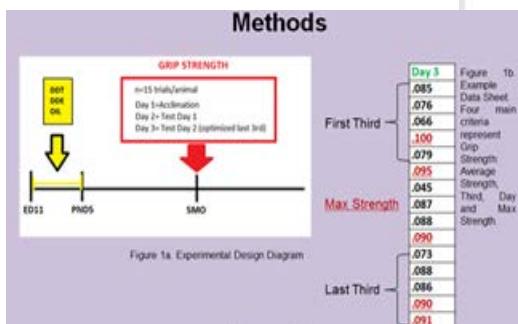


Figure 1. Animals were maintained on a 12:12 light dark cycle and maintained on Purina mouse chow. MC-F-7 mouse mammary tumor cells (ATCC) were cultured in DMEM with 5% fetal bovine serum (Gibco-BRL) at 95% CO₂ in T25 coated flasks (Falcon). Cells were collected at 50% confluence and diluted to 10⁶ cell/ml in physiological saline (Hyclone). 0.1 ml of the cell suspension was injected subcutaneously into 5 regions of the back of nude mice. Tumors were allowed to develop for 30 days, and measured. Mice were separated into untreated, sham IP injected, high dose Compound-X (7 micrograms/gram wt) and low dose (2 micrograms/gram wt) groups, and then treated for 30 days. Animals were timed to judge their total daily time spent in grooming activities (Switzman Rodent Depression Test; Switzman et al. 1994), to assess possible depressive effects of the treatment. After 30 days, tumors were measured across their greatest width, both externally and after harvest. Results were analyzed using a student's T-test.

MATERIALS

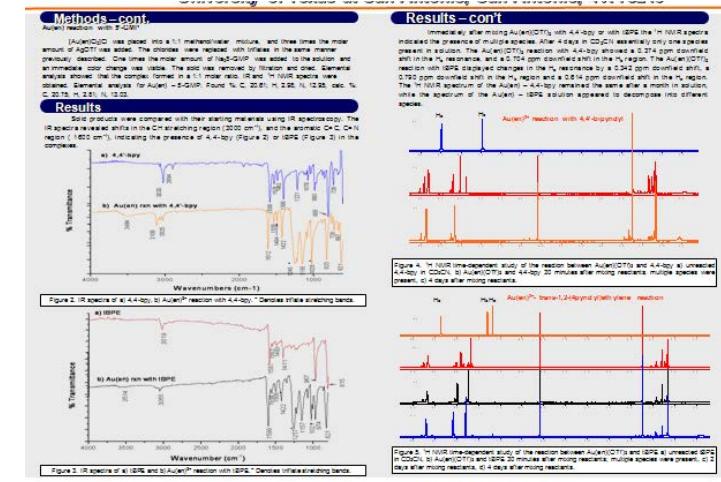
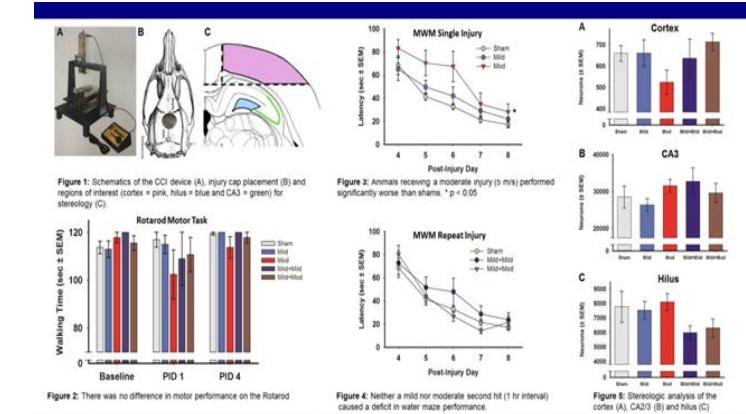
Coating process by Sol-gel methods: Commercially pure titanium (Ti) disks of (15 mm diameter and 2.0 mm thick) were used as substrates. All disks were wet ground with 240, 400 and 600 grit silicon carbide papers, followed by ultrasonic degreasing using acetone and ethanol for 10 minutes each. Deionized water was used for rinsing the disks between applications of each solvent. A passivation procedure was then conducted by exposing the Ti disks to a 40% volume nitric acid solution at room temperature for 30 minutes (ASTM F86-91).

Prior to coating on the passivated Ti surfaces, hydroxyapatite (HA) and 1 wt% silver (Ag)-doped HA (HA-AG) sol were produced. The HA sol was prepared by reacting calcium nitrate tetrahydrate [Ca(NO₃)₂·4H₂O] with methyl alcohol to produce calcium precursors. Phosphorus precursors were also prepared by reacting triethyl phosphite [(OC₂H₅)₂P] in 0.03 ml acetic acid (CH₃COOH). The two precursors were then mixed and 0.1 mol of DCCA (Drying Control Chemical Additive) was added to the mixture. All reactions were carried out in argon atmosphere. Similar to the HA sol, AgHA1.0 sol was produced by mixing the calcium and phosphorus precursors with 1.0 wt % silver nitrate (AgNO₃) and 0.1 mol DCCA. AgNO₃ was chosen for Ag doping because of the easy decomposition of nitrates during heating.

The prepared HA and HA-AG sol were then coated on passivated Ti surfaces by spin coating at 5,000 rpm for 50 seconds. The coated-Ti surfaces were immediately dried at 70°C for 12 hours, followed by a heat treatment at 650°C for 3 hours. The HA-coated surfaces were used as controls in this study. All samples were autoclaved prior to materials characterization and all culture experiment.

Results

- Largest section
- Vary with field
- Often two middle columns
- Summarizes the data and reports results of statistical tests and analyses (- or +)
- Draw implications and considerations
- Don't present raw data
- Make Image-based; use few words
- Maximize use of Figures
 - Make them simple
 - Must be easily seen
 - Make all lines wide enough
 - All text large enough!
 - Consistent axes across poster
- Minimize use of tables
 - Difficult to grasp quickly
- Use figure legends/captions as text
- Put text near figure it's describing
- ~1 paragraph per image/image group



Conclusions/Discussion

- Or discussion or summary
- Very few words
- Bullets good
- Bigger font if needed
- *Summarize “take home” results
 - Interpret the meaning or implications of your results
 - Mention any alternative explanation for results or unanticipated results
- *How did hypothesis work out?
- *Tie back to real world problem
- *Why Important/Implications
- Aim for:
 - Reasonable conclusions were given and strongly supported with evidence
 - Conclusions were compared to hypothesis and their relevance in a wider context was discussed

Conclusions

- We have adapted a group II intron-based system for efficient targeted mutagenesis of *F. tularensis*
- This system is effective and efficient across *F. tularensis* subspecies: *tularensis*, *holarsctica*, and *novicida*
- This system was used to successfully disrupt *blaB* found in single copy in the *F. tularensis* genome.
- This system was used to successfully disrupt both copies of the duplicated *IgIC* gene in a single manipulation.
- Targetrons should be a valuable genetic tool for the dissection of *F. tularensis* pathogenesis.

This study was supported by NIMH F31AI097956 to KDK and NIMH GM060425 to SAR.

SUMMARY AND CONCLUSIONS

In this study, x-ray diffraction analysis of Ag-doped HA thin film by sol-gel method indicated peaks corresponding to HA. Contact angles for HA-Ag surfaces were observed to be significantly lower when compared to HA surfaces. *In vitro* bacterial adhesion study indicated a significantly reduced number of *S. enterica* and *S. aureus* on HA-Ag surface when compared to HA surface, whereas significantly reduced adhesion of viable *S. aureus* was observed on HA-Ag surface when compared to Ti and HA surfaces. Additionally, no significant difference of osteoblast activity was observed on three different surfaces tested. Overall, it was concluded that the 1% Ag-doping on HA surfaces were non-toxic to osteoblast cells. Additionally, it was also concluded that the 1% Ag doping was effective in reducing bacterial adhesion.

References/ Literature Cited

- Include sources/resources that supported your work
- If someone's work is cited (usually in introduction), you must include a reference
- Generally “short” (title optional)
- Can use smaller font if needed

References

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2. Engle-Friedman, Dr. Mindy. "Baruch College of the City University of New York Waste Audit Report." *YRG Sustainability*, 14 June 2010. Web.
3. Divya Dayal, Macaulay Honors Intern of the Baruch College Sustainability Task Force. Interview conducted by Aaron Lam
4. "NYCWasteLess." *NYCWasteLess*. The City of New York. Web. <<http://www.nyc.gov/html/nycwasteless/html/home/home.shtml>>.
5. Survey Data from Chinatown, Flushing, and Fresh Meadows

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 1. Merced County. (2007). Merced County Supervisional Districts. Retrieved September 20, 2008, from <http://www.co.merced.ca.us/bos/district3.html>.
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3. Fujita, M.; Yazaki, J.; Ogura, K. *J. Am. Chem. Soc.* 1990, 112, 5645

Acknowledgements

- Acknowledge the faculty and staff who supported you.
- Thank people
 - Mentor
 - Research group
 - Technical assistance, etc.
- Reveal possible conflicts of interest
- Identify funding utilized
 - CAMP, LSAMP-NSF, NIH, etc.
- Font can be smaller than rest of text

Thank You!



Acknowledgements

We would like to thank Mr. Angus Rhododendrum and Suzanne McPerkins for their technical assistance.

Funded by NIH Grant #94-90082, the MBRS-RISE program (NIGMS #22209587), and the American Tobacco Association.

Dr. GP Taylor is a paid consultant for the Amelloron company, which has been licensed to develop Substance-X as a chemotherapeutic agent.

Acknowledgements

National Institute of Health (NIH-SCORE program, Grant No. GM-08194)

Partially funded by NIH/NIGMS MBRS-RISE GM6065

Acknowledgements

❖ We would like to thank:

- ❖ Our mentors Dr. Stergios Roussos and Dr. Maria G. Pallavicini for their support during the long and strenuous journey of establishing ITCH.
- ❖ All participating ITCH members whose hard work has made this organization a possibility.
- ❖ All community leaders, community professionals, and UCM faculty whose devoted time and patience has been greatly appreciated and has helped with the establishment of ITCH.



High Resolution Reconstructions of Sea Surface Temperatures from Pacific Geoduck Growth Increment Chronologies

Matthew J. Stuckey¹ & Bryan A. Black²

¹University of California, Berkeley, Berkeley CA 94720, USA.

²Oregon State University, Hatfield Marine Science Center, Newport OR 97365, USA.

National Science Foundation Research Experience for Undergraduates

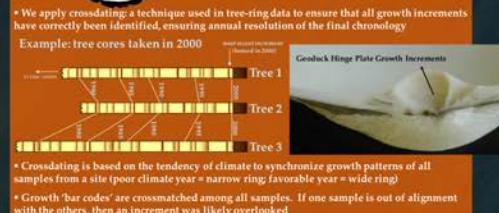
Hatfield Marine Science Center, Oregon State University

March 2008 Ocean Sciences Meeting, ASLO

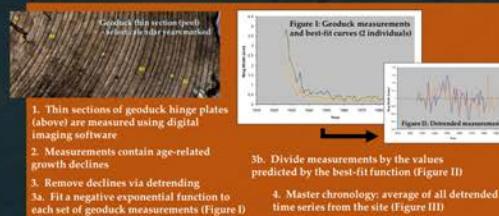
Introduction



Methods



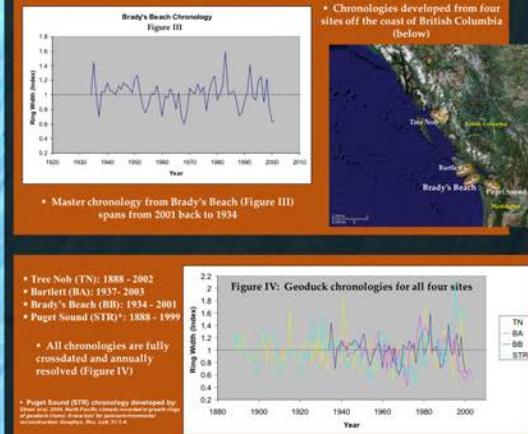
- Crossdating is based on the tendency of climate to synchronize growth patterns of all samples from a site (poor climate year = narrow ring; favorable year = wide ring)
- Growth 'bar codes' are crossmatched among all samples. If one sample is out of alignment with the others, then an increment was likely overlooked



Abstract

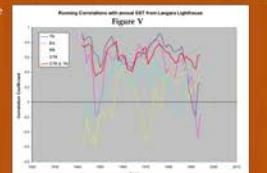
We demonstrate the potential for reconstructing sea surface temperatures along coastal British Columbia, Canada, using four chronologies developed from the growth increment widths of Pacific geoduck clams (*Panopea abrupta*). The four geoduck chronologies range from the southernmost to northernmost borders of British Columbia and were developed using standard tree-ring (dendrochronology) techniques, including crossdating. Although each geoduck chronology significantly correlated with local records of sea surface temperatures (SST), correlations were unstable over time. In every chronology, the relationship with SST would occasionally dissolve for a period lasting approximately ten years. The timing of these climate-growth breakdowns was inconsistent and varied among the chronologies. For any one chronology, inconsistent climate-growth relationships represented a significant complication for developing accurate SST reconstructions. However, when geoduck chronologies were combined via simple averaging, irregularities in climate-growth relationships canceled out one another to yield strong and highly stable SST reconstructions. Final SST reconstructions captured more than 60% of the variance in the instrumental record and extended more than 120 years, capturing the historical range of variability and providing context for current climatic trends.

Results



Discussion

- Sea surface temperatures are recorded at nine lighthouses off the coast of British Columbia



- Geoduck chronologies strongly correlate with SST records
- Potential tool for SST reconstructions
- However, correlations with SST are inconsistent over time (Figure V)
- Solution: average multiple chronologies
- Cancels out irregularities and forms more stable climate-growth relationships
- Example: Average of Tree Nob and Puget Sound is much more stable (Figure V)

- Due to geoduck longevity, sea surface temperature reconstructions substantially predate instrumental records (Figure V)

- Example: Average of Tree Nob and Strom chronologies explains 50% of the variance in the SST record at Langara Lighthouse (left), using linear regression



Acknowledgements

- Many thanks to...
 - HMSC & OSU for hosting the REU program
 - NSF for funding this project under award OCE-0648515
 - Pacific Biological Research Station of the Department of Fisheries and Oceans Canada for providing our geoducks
 - Iitching Cheung, Dr. George Brattell, and many others at HMSC for shaping the REU experience
 - Ross Koenig for assistance with the geoduck and Barnacle chronologies
 - Avi Strom for developing the Puget Sound chronology
 - Dr. Bryan Black for his ongoing mentorship and tremendous help with this project
- For more information, please contact Matt at mstuckey@berkeley.edu or Bryan at bryan.black@oregonstate.edu

Examples of Excellent Posters

Does Perinatal Exposure to DDTs and the Development of Glucose Intolerance Promote Skeletal Muscle Deficiency?



Ciara Main¹, Michele La Merrill Ph.D²
Department of Animal Science¹, Department of Environmental Toxicology², University of California, Davis



Abstract

The once ubiquitously used pesticide DDT and its metabolite, DDE (together, DDTs) have been an environmental health concern for many decades. Recent epidemiological and mechanistic data link DDT exposures with devastating diseases such as obesity, hypertension, and components of Type 2 Diabetes. Our work surrounds perinatal exposure of DDTs and adult phenotyping. C57BL/6J mice were exposed to DDTs from embryonic day 11 to postnatal day 5, raised on normal chow, and switched to high fat diet (HFD) at 4 months to initiate obesity. Three months after exposure, dams exposed to DDE during pregnancy were glucose intolerant, while their female offspring displayed elevated fasting insulin. Disruptions in peripheral glucose utilization prompted us to explore whether tissues that rely heavily on glucose uptake were displaying a phenotypic defect. One month after being put on HFD (5 months after exposure), we measured muscle strength. To assess muscle deficiency, we tested forelimb grip strength (GS) using Chatillon Machinery Grip Strength Machine (Largo, FL). GS was tested over three days with 15 trials/day. On days two and three, overall grip strength, max strength, and first and last third of each trial were analyzed. Dams showed a difference in strength between days two and three, however F1 offspring had no significant change between treatment groups. Although, we did not find conclusive evidence that DDTs impair skeletal muscle function, further research is needed to examine potential indirect effects that DDTs may have on skeletal muscle.

Introduction

- DDTs are apart of a group of toxicants named Persistent Organic Pollutants (POPs) that accumulate in animal tissues.
- DDTs are a risk factor for glucose intolerance.
- One symptom to glucose intolerance is impaired glucose uptake in tissues.
- There is no prior evidence suggesting DDTs directly effecting Grip Strength in skeletal muscle.

Hypothesis

Perinatal exposure to DDTs causes impaired glucose uptake in skeletal muscle resulting in a decrease in GS.

Methods

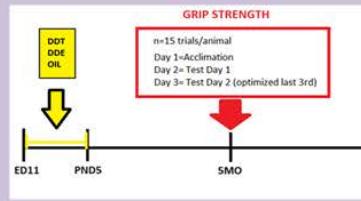


Figure 1a. Experimental Design Diagram



Results

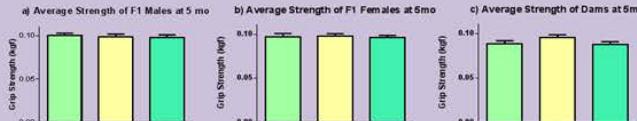


Figure 2. Average Grip Strength effects of F1 male (a), F1 female (b) and F0 dams (c) when separated by treatment.

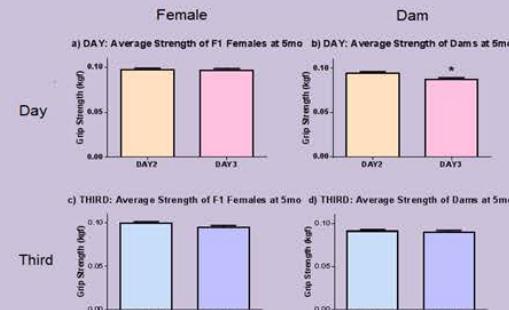


Figure 3. Data from F1 female (left column) and F0 dam (right column) average GS at 5mo in respect to Day (top row) and Third (bottom row) criteria.

Results continued

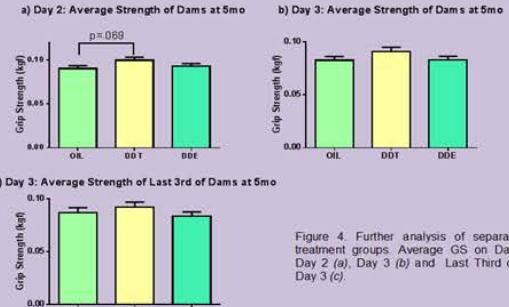


Figure 4. Further analysis of separate treatment groups. Average GS on Dam Day 2 (a), Day 3 (b) and Last Third on Day 3 (c).

Max Strength

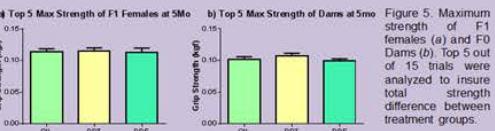


Figure 5. Maximum strength of F1 females (a) and F0 Dams (b). Top 5 out of 15 trials were analyzed to insure total strength difference between treatment groups.

Conclusion

- At 5 mos, DDTs did not effect GS regardless of sex, exposure type, or GS criteria (Avg. GS, Day, Third, & Max Strength).
- Dam GS on Day 3 (Fig 3b) decreased compared to Day 2.
- Given smaller SE and CV (data not shown) we conclude that GS measured on Day 2 is more robust than Day 3 due to possible decrease in endurance of Dam Day 3.
- Optimizing the Last Third on Day 2 is the best strategy to collect Grip Strength.

Acknowledgements

Extreme gratitude to Michele La Merrill Ph.D for giving me this opportunity to work in her lab. She has encouraged me to build novel skills as well as add upon existing. McNair Scholars Program and California Alliance for Minority Participation (CAMP) Program for providing me the resources for my future career in research.

Examples of Excellent Posters

Expression, purification, and crystallization of recombinant mouse phospholipase c-zeta (PLC- ζ)



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ABSTRACT

The aim of this study is to express and purify recombinant PLC- ζ protein for structure identification through X-ray crystallography. To date, there is no available empirical data of the 3D structure of PLC- ζ . The identification of the structure is crucial as it presents information that will facilitate understanding of the protein mechanism and regulation, both of which remained unknown. Bioinformatic analysis was also utilized to draw initial structural information, specifically on the domain differences of PLC- ζ , and empirically determined structure PLC- δ .

INTRODUCTION

Phospholipase C- ζ (PLC- ζ), a member of phospholipase C family, was identified as the sperm factor responsible for activating oocytes, and thereby causing fertilization.

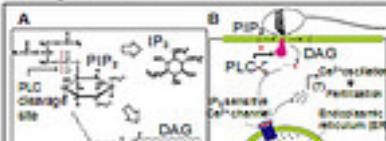


Figure 1. Enzymatic action of PLC- ζ . (A) Hydrolisis of PIP₂ by PLC- ζ (released from sperm) produces DAG and IP₃. (B) In turn, IP₃ activates Ca²⁺ channel of ER to release calcium. This hypothesis leads to Ca²⁺ oscillation and eventually fertilization.⁴

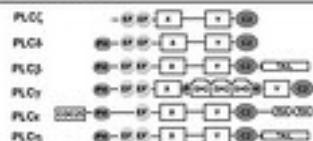


Figure 2. PLC Domain Organization. PLC- ζ consists of EF-hand domain, catalytic (X and Y) domain, and C2 domain. These domains are also found in other PLC isoforms. PLC- δ showed closest resemblance to PLC- ζ .⁴

Bioinformatic analysis through sequence alignment and homology modelling revealed that the calcium binding region of C2 domain as well as the catalytic Y-region of PLC- ζ were expected to be significantly different from empirically determined PLC- δ .

EXPERIMENTAL RESULTS



Figure 3. Molecular cloning of PLC ζ 124 construct. (A) Two step PCR amplification successfully produce a PLC- ζ construct with 6-HIS and 3C protease cleavage site (1813 bp in size). (B) Construct was ligated into pET102/D-TOPO vector. This is validated by restriction digest using *Ccl1*. Vector alone (1) showed a lower band compared to vector with the construct(2).

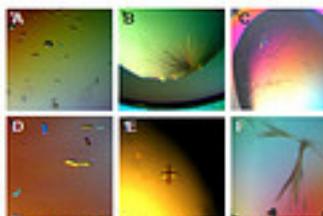


Figure 5. Crystallisation of PLC ζ 124 Construct. Six different screening methods were found to be suitable for crystallizing the protein. Crystals were confirmed to be protein due to birefringence characteristic under polarised light. Protein crystals A-E were needed to be optimised to obtain larger crystal. Protein crystal F was tested for X-ray diffraction. Preliminary analysis, however, revealed that X-ray diffraction pattern was hindered by presence of high salt concentration.

EXPERIMENTAL PROCEDURE

PLC ζ 124 construct was generated using two-step PCR to incorporate 6-HIS and 3C protease recognition site. Construct was ligated into pET102/D-TOPO vector and transformed into *E. coli* BL21(DE3). Protein expression was induced using IPTG. Bacterial lysis was carried out using French Press. Protein construct was captured using Nickel beads and cleavage of the protein from the tags was completed by 3C protease. Further purification was carried out using RP-HPLC (ion-exchange and gel filtration chromatography). Crystallization of protein was carried out using sitting drop vapor-diffusion method.

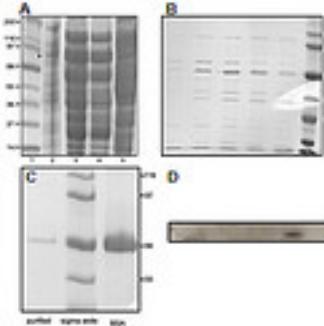


Figure 4. Protein expression and purification. (A) Molecular weight marker (lane 1). Protein bands after IPTG induction (lane 2). Protein construct migrated at ~83 kDa. Nickel beads were used to capture protein (lane 3) and the beads were washed with high salt concentration (lane 4) to remove contaminants (lane 5). (B) Fractions collected after cleaved protein (by 3C protease) passed through RP-HPLC-ion exchange method. Bands migrating at around 66 kDa (which corresponds to PLC ζ 124 protein) are found. (C) Further purification through RP-HPLC gel filtration method to obtain purified sample. (D) To verify that indeed the protein band is PLC- ζ , Western blot was employed using antibody specific to XY linker.

CONCLUSION

- It was predicted from the bioinformatic analysis that PLC- ζ will fold in the same general topology as PLC- δ (without PH domain).
- Specific differences were predicted to be in the Y-region of catalytic domain and C2 domain.
- This hypothesis, however, was not tested as X-ray diffraction data collection failed. This was due to presence of high salt concentration. Future study may need to alter buffer systems to obtain this structural data.
- The recombinant mouse PLC- ζ was successfully expressed, purified and crystallized. However, the expression levels is low.
- It was assumed that the protein was catalytically active in bacterial cell and overproduction caused toxicity and metabolic stress.
- To obtain higher protein expression, different vector system and bacterial strain maybe used.
- The ultimate aim is to reveal the 3D structure of human PLC- ζ . However, the expression of the human PLC- ζ was much lower. It is possible though to construct a more accurate model if an empirical 3D structure of mouse PLC- ζ was determined and used as a template.

ACKNOWLEDGEMENTS

I would like to thank Dr. A. Rossbach for the antibody used in Western blotting, Dr. LO D'Cruz for the PLC ζ 124 construct, 3C protease and his supervision, Mr. Peter Wilson for technical support.

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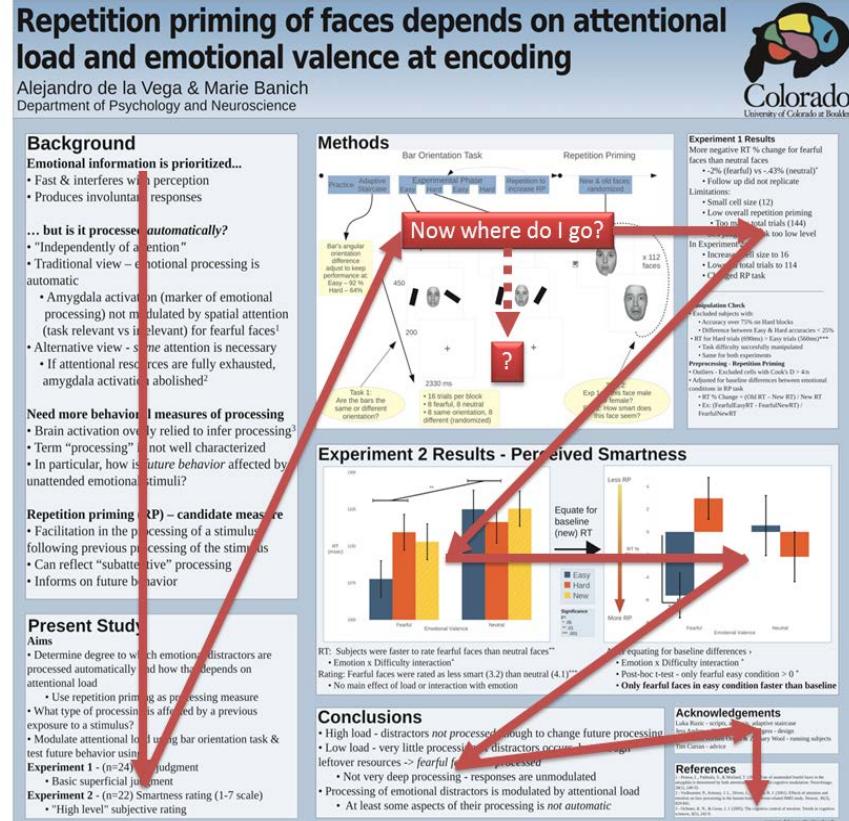
First Contact

- Stand to left of poster (where start reading)
- Take initiative
- Smile, but stay near poster
- If they come closer
- Say, “Hello” and shake hands
- Give name. Get their name.
- Give level, and university (UC Davis)
- Ask if they’d like “you to walk them through your poster”
 - YES? Then GO!
- This is work that I performed this summer in the ___ program in the laboratory of Dr. _____ at UC Davis.
- (Optional) Ask if they are familiar with this field of research
 - No- More introduction, careful with acronyms
 - Yes- Can go more quickly through intro



The Flow of Things

- Start with Intro that will catch them
 - No pointing if you have no figure!
- Move to Methods
 - Briefly summarize
- Move to Results
 - Longest section
 - Indicate at beginning if did not work
 - Walk thru all figures
- Transition to Conclusions
- Say Conclusions
- Acknowledgements (optional)
- Any Questions?



Project Progress

Project updates (I)

- Classification of distribution power system faults
 - Haoyuan
- Accelerating the Dimer Method with Machine Learning Techniques
 - Liubin

Project updates (II)

- Neutron Events Detection Using Clustering
 - Rebecca and SuAnn
- Using Different Clustering Methods on Single Cell RNA Sequencing Gene Expression Data to Determine Genes of High Importance For Human and Mouse Cells
 - Angelica

Project updates (III)

- Impact of soil moisture in wildfire simulations
 - Kae
- Freesound General-Purpose Audio Tagging
 - Pengxiag and Bohan
- Performance Comparison of Different MPIs using Hatchet
 - Ian

Project updates (IV)

- Authorship Identification Using N-Gram Feature Classification
 - Austin
- Medicaid Data Set from Delaware
 - Elizabeth and Aileen
- Stock Prediction
 - Abhijeet

Project updates (V)

- Detecting Trends in Twitter Health News
 - Nasib and Burcum
- Measurement of probable vulnerability of self-harm in different demographic using CDC survey data
 - Mohammad
- A Deeper Look into Scalable Methods for Creating Food Groups Using NHANES Dataset
 - Samuel