RECOGNIZING OUTSTANDING UNDERGRADUATE RESEARCH

2018 Research Day at the Capitol

STUDENT ORIENTATION SESSION

2018 Research Day at the Capitol

Congratulations for being selected to represent your institution at the

23rd Annual Research Day at the Capitol!

Event Sponsors:

Oklahoma NSF EPSCoR

The National Science Foundation

Oklahoma State Regents for Higher Education

What is Research Day at the Capitol?

- Annual event, sponsored by:
  - Oklahoma State Regents for Higher Education
  - The National Science Foundation (NSF)
  - Oklahoma Established Program to Stimulate Competitive Research (OK NSF EPSCoR)

- To celebrate the excellent undergraduate student research being conducted on Oklahoma's college and university campuses

- A chance to inform Legislators and the public about undergraduate student research

2018 Research Day at the Capitol

LET'S HEAR ABOUT YOU! GIVE US THE ELEVATOR PITCH

Tell the Group (in 45 seconds or less)

- WHO YOU ARE
- WHAT INSTITUTION YOU'RE REPRESENTING
- WHAT YOU'RE RESEARCHING
- WHAT THE SOCIETAL IMPACT IS

Remember…not everyone is familiar with your area of expertise, so don't use area-specific lingo or jargon.

Research Day at the Capitol

Research Day at the Capitol

2018 Research Day at the Capitol

TWO DAYS OF ACTIVITIES – MARCH 26 & 27

March 26 (Hyatt Place Hotel)

- Formal judging: poster & oral presentations

March 27 (State Capitol Building)

- Posters presented on 4th Fl. Capitol Rotunda
- Awards ceremony

Students must participate in all activities to retain the $250 stipend and qualify for prizes.

2018 Research Day at the Capitol

YOUR STIPEND FUNDING

You can expect delivery of your $250 stipend check within approximately 3-4 weeks. Call our office it is hasn't arrived by Dec. 13 and we'll attempt to track it.

- Funds are to cover your travel to/from OKC and for costs related to your poster printing & display (easel, board, etc.).
- Checks will be mailed to your permanent address, which may/may not be your university address.
- Checks will be issued from "OKLAHOMA STATE UNIVERSITY," NOT "OK EPSCoR."
- OSU students' checks will be processed through the OSU Bursar's Office.

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CASH PRIZES: FOR THE TOP 7 PRESENTERS

The following prizes will be awarded:

Grand Prize: $500 cash prize + $4,000 summer research internship
$2,500 award to the sponsoring college/university lab to offset expenses of hosting the internship

1st, 2nd, and 3rd Place Prizes will be awarded in each of two categories: Regional/community colleges (3 awards/1st, 2nd, 3rd)
Research-intensive campuses (3 awards/1st, 2nd, 3rd)

1st Place: $500 cash prize (1 ea: regional & research-intensive)
2nd Place: $250 cash prize (1 ea: regional & research-intensive)
3rd Place: $250 cash prize (1 ea: regional & research-intensive)

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HOW YOU ARE JUDGED

3 - 4 Judges: WELL educated, but not necessarily experts in your field of study.

You will be judged on the following components:

- ❖ Abstract
- ❖ Oral Presentation
  - Timed, 3-minutes, in front of a panel of judges
  - 2-minutes of timed Q&A
- ❖ Poster

Refer to the sample judging sheet in your packet for scoring details.

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JUDGING CRITERIA (FROM THE SAMPLE SCORE SHEET)

The following judging criteria are used, with a 1-10 scale for each item:

- Abstract
  Format, clarity, societal impact, objective of study, results, conclusions, etc.
- Scientific presentation
  Clear purpose, hypothesis, background information, results, impact, further study expected
- Student's ability to explain the project
- Visual appearance
- Clarity for general audiences
- Societal impact statement
- Overall

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ABSTRACT: REVISION DEADLINE FEBRUARY 5, 4 P.M.

Judges will score your abstract as part of your cumulative score. If you wish to alter or edit the abstract that was originally submitted, you must submit your final, revised abstract in MS Word format prior to February 5th at 4 p.m.

- MS Word format, no PDFs accepted
- Maximum 350 words
- If images are used they will detract from the available word count; image files must be submitted separately (not only embedded in the document).
- Avoid excessive scientific jargon, but don't oversimplify
- Must be the work of the student
- See the provided sample judging sheet for scoring criteria
- Be sure you receive a confirmation of receipt from me

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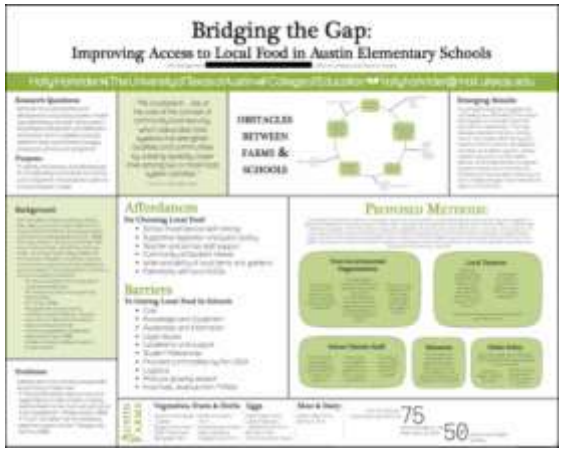
THE POSTER

- The poster: crucial to your success
- "Best practices" in poster development will be addressed later in the presentation.



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MONDAY, MARCH 26 * HYATT PLACE HOTEL, OKC

11:00 a.m – 5:00 p.m. Poster & Oral Presentation Judging

- Individual timeslots will be provided in advance.
- Arrive at designated time.
- Bring your poster with you, mounted on a firm board.
- Check in at the EPSCoR table.
- Poster number will be provided at check-in.
- Place the number in the top/right corner of your poster.
- Wait outside the judging room for your turn to present.
- Students will enter the judging room one-at-a-time.
- Take your poster in with you.
- **IMPORTANT!!** An easel will be provided in the judging room. However, YOU must bring your own easel to the Capitol the following day.

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POSTER AND ORAL PRESENTATION JUDGING

Oral Presentation: 3 minutes (timed)

- Walk in- SMILE, introduce yourself, be confident, and walk them through what you have done - using your poster as a guide or reference.

Q & A: 2 minutes (timed)

- Anything on your poster is eligible for questioning so BE FAMILIAR with all components.
- Questions are usually to re-affirm or clarify something about your presentation.
- Kinds of questions - Procedural, social impacts, future aspirations

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- ❖ After Q&A: Exit the room with your poster.
- ❖ Leave the number on your poster for Tuesday.
- ❖ You are free for the rest of the day.

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PRESENTATION PREP & SUGGESTIONS

- The best way to improve your presentation skills is to present.
- Record yourself presenting and play back your recording to notice and fix your mistakes.
- Practice presenting to a non-science friend and listen to their feedback on your presentation.
- Practice presenting in an empty room using the volume you plan to speak at and hand gestures (pointing to figures/text on poster).
- Maintain natural eye contact with your audience in order to keep them engaged.
- Emphasize the importance of your societal impact. Make them feel that your scientific findings are important.
- What if you're asked a questions that you don't know the answer to? Do NOT make up an answer—it's better to say you don't know.

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ADDITIONAL PRESENTATION SUGGESTIONS

- Time yourself to make sure you can present in the 3-minute timeframe.
- If you forget your next point do not panic. Calmly collect yourself and keep moving.
- Smile and be warm to the judges. They are spending their time listening to you talk. Be gracious.
- Repetition is the key to presentation success.

Research Day at the Capitol

SUGGESTIONS FROM THE JUDGES

- Review sample judging criteria sheet
- Review your abstract and make sure it's accurate; use the space that you have been allotted & revise if necessary
- Talk loud and project your voice
- Pay close attention to societal impact and research objective
- Answer, "What have you accomplished with your research?"
- Statistics are good—provide proof of outcomes

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SUGGESTIONS FROM THE JUDGES

- Focus on what you contributed in regards to the research. Don't claim to have done it all if that's not the case. Toot your horn if it's applicable!
  "With assistance I…."
  "In collaboration with my faculty mentor I….."
  "I explored ____ with the grad assistant on the project."
  "I independently performed……"
- Avoid jargon in oral presentations; clarity for general audiences should be considered
- Societal impact statement should be included on the poster and also in the oral presentation

2018 Research Day at the Capitol

HOTEL ACCOMMODATIONS (REQUEST DEADLINE FEB. 5)

EPSCoR will provide lodging on the evening of Monday, March 26 for student participants who live outside the OKC metro area and who have requested lodging prior to the February 5th deadline.

- Conference hotel: Hyatt Place Hotel
  The EPSCoR office will book the room for you and pay the hotel directly for the room charge.
- Hotel will require a credit/debit card from students at check-in to cover any incurred incidental charges.
- A guest may stay in the room with you at no additional charge.
- Email me no later than Feb. 5 to secure a room; a signup sheet is available today (indicate one bed or two in the room).
- Confirmation numbers will be issued to you in February.
- If a room is booked on your behalf and is not used, you/your institution will be responsible for the charges.

2018 Research Day at the Capitol

TUES., MARCH 27 CAPITOL POSTER SESSION

2018 Research Day at the Capitol

TUES., MARCH 27 TIMELINE: CAPITOL POSTER SESSION

6:30-7:30 a.m.	Breakfast for students staying at the Hyatt *(Free for guests, ask front desk for location)*
8:00-8:30 a.m.	Students arrive at Capitol, 4th Floor *(Check-in at EPSCoR table; setup posters)*
8:30-11:15 a.m.	Posters on exhibit, 4th Floor *(Students greet Legislators & Capitol guests)*
11:30 a.m.	Awards Ceremony, Blue Room, 2nd Floor
12:30 p.m.	Adjourn & take down posters

2018 Research Day at the Capitol

TUES., MARCH 27 ARRIVAL INFORMATION

- Give your self plenty of time—it's hard to navigate around the Capitol streets & parking lots in the dark (and it's dark before 8 a.m.)
- Park in the approved visitor parking area
  (I will send you a map)
- Go through security (no knives, etc.) & take elevator to the 4th floor
- Check in at the EPSCoR booth & receive name badge & abstract book
- Set up your poster
- **Everyone must be set up and ready to go by 8:20 a.m.**

2018 Research Day at the Capitol

MARCH 27 CAPITOL POSTER SESSION

A six-foot table covered with a white, floor-length tablecloth will be provided for you.

You are required to bring:

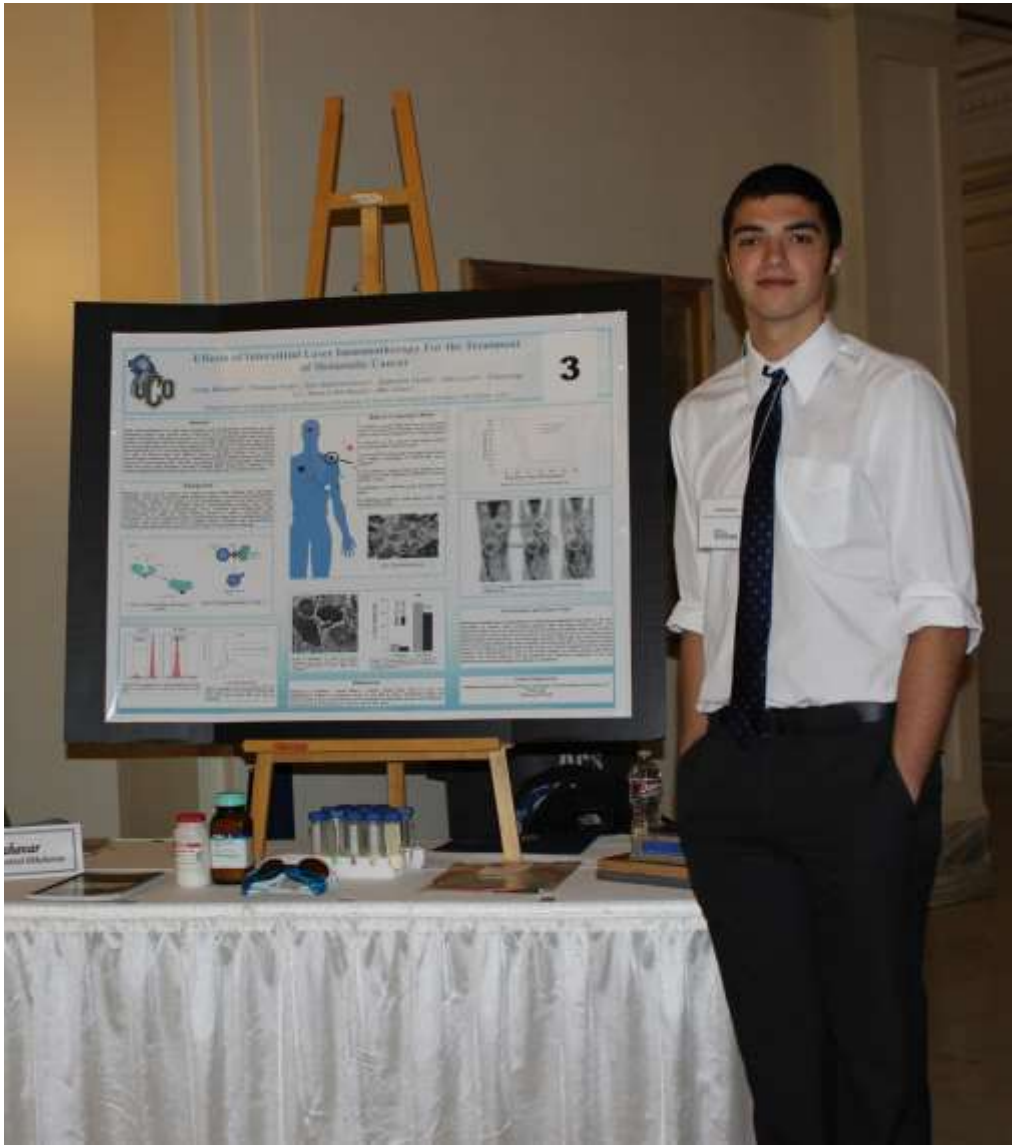
- Photo ID
  (May be requested by security at the Capitol entrance)
- Your poster
- Firm board backing for your poster
- Easel
- Tacks, Velcro or other attachment materials
- Your poster number that was provided the previous day

You are highly encouraged to:

- Bring hands-on demonstration materials

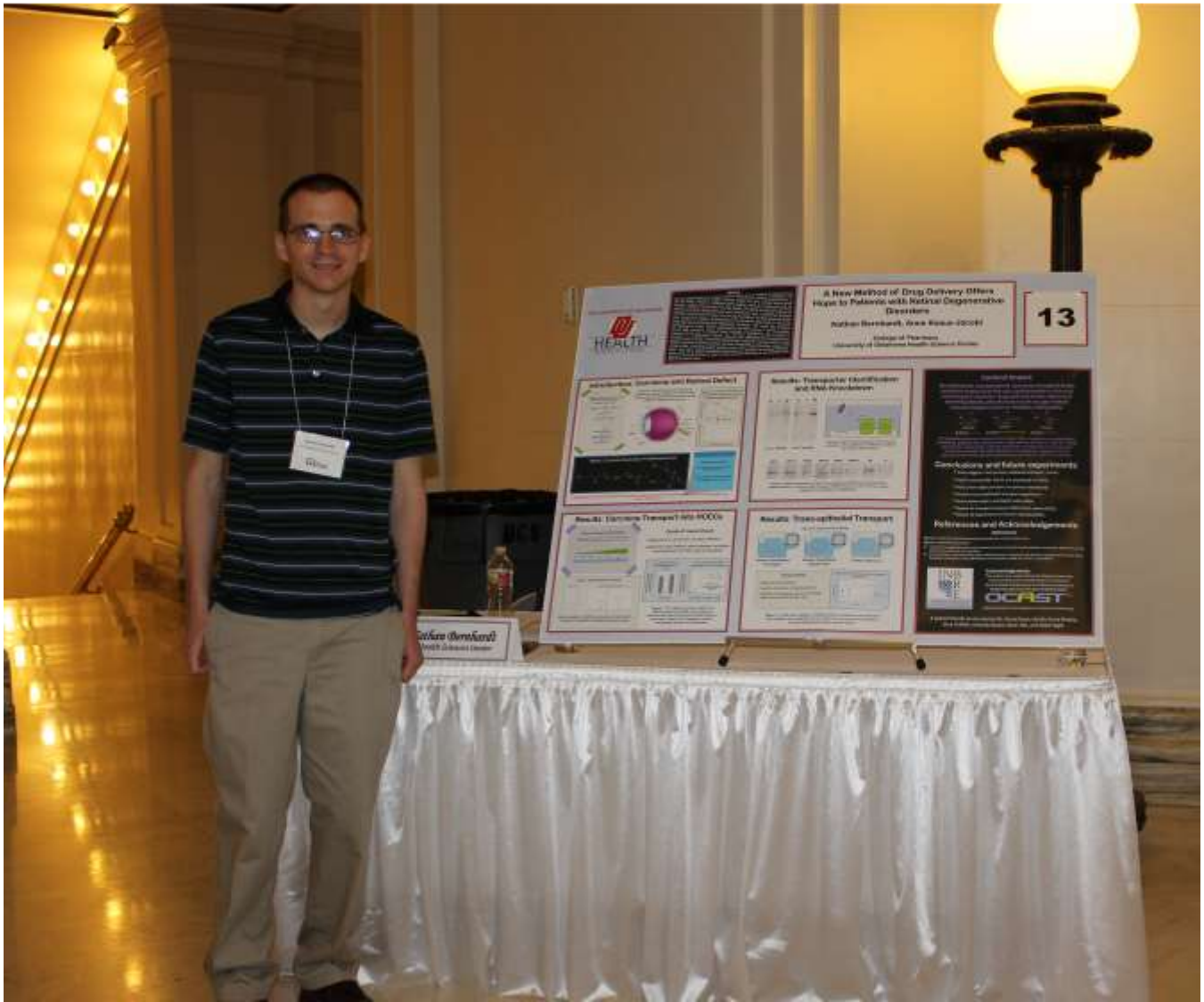
March 27 * At the Capitol

BRING HANDS-ON DEMONSTRATION MATERIALS



*March 27 * At the Capitol*

DRESS PROFESSIONALLY; A NEW SUIT ISN'T NECESSARY



Research Day at the Capitol

APPROPRIATE DRESS

*March 27 * At the Capitol*

DO NOT FORGET YOUR EASEL AND FIRM BOARD!

March 27 * Day of the Event

SHARE YOUR WORK: RDC JUDGES, LEGISLATORS & CAPITOL VISITORS

*March 27 * At the Capitol*

THE LEGISLATORS

- Identify your home and school Representatives and Senators (may be different) www.oklegislature.gov
- Remember: Use layman's terms & outline how your research affects and/or benefits his/her constituents!
- Not everyone will receive a citation, but we make a recommendation and provide details to encourage it.
- Grab a photographer.

2018 Research Day at the Capitol

TIMELINE OF IMPORTANT DATES

Nov. 12, 2017 – March 25, 2018	Students prepare scientific posters & oral presentations
Monday, February 5, 2018	Students' revised abstracts and lodging requests are due
Monday, March 12, 2018	Online registration closes
Monday, March 26, 2018	Poster/oral presentation judging 11 a.m. – 5:00 p.m. Hyatt Place Hotel, Oklahoma City *Student Participation Mandatory*
Tuesday, March 27, 2018	Posters on Exhibit 8:30 – 11:15 a.m. State Capitol, Oklahoma City *Student Participation Mandatory*
Tuesday, March 27, 2018	Awards Ceremony 11:30 a.m. – noon Blue Room, State Capitol, OKC *Student Participation Mandatory*

All March 26-27 activities are mandatory for student researchers; registered guests are invited to participate in all Capitol activities on the 27th.

2018 Research Day at the Capitol

POSTER PREP: A QUICK REFERENCE GUIDE

Purpose of your Research Poster: Disseminate research findings and progress to Legislators, the public, and your peers

- Will not be a cut-and-paste version of your abstract
- Visually communicates a “take-away message”
- Spotlights your most important ideas, points, findings
- Serves as an interface between your research results and your oral presentation

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❖ *Posters must be the work of the student researcher.*

2018 Research Day at the Capitol

POSTER PREP: A QUICK REFERENCE GUIDE

- PowerPoint is recommended for your poster design

- Before you start
  - Check with your print shop regarding size and color constraints that may apply
  - Average size 48”x36”
  - Set the page size (in your (program) to match the final print size
  - Ask your mentor for advice regarding where to print your poster

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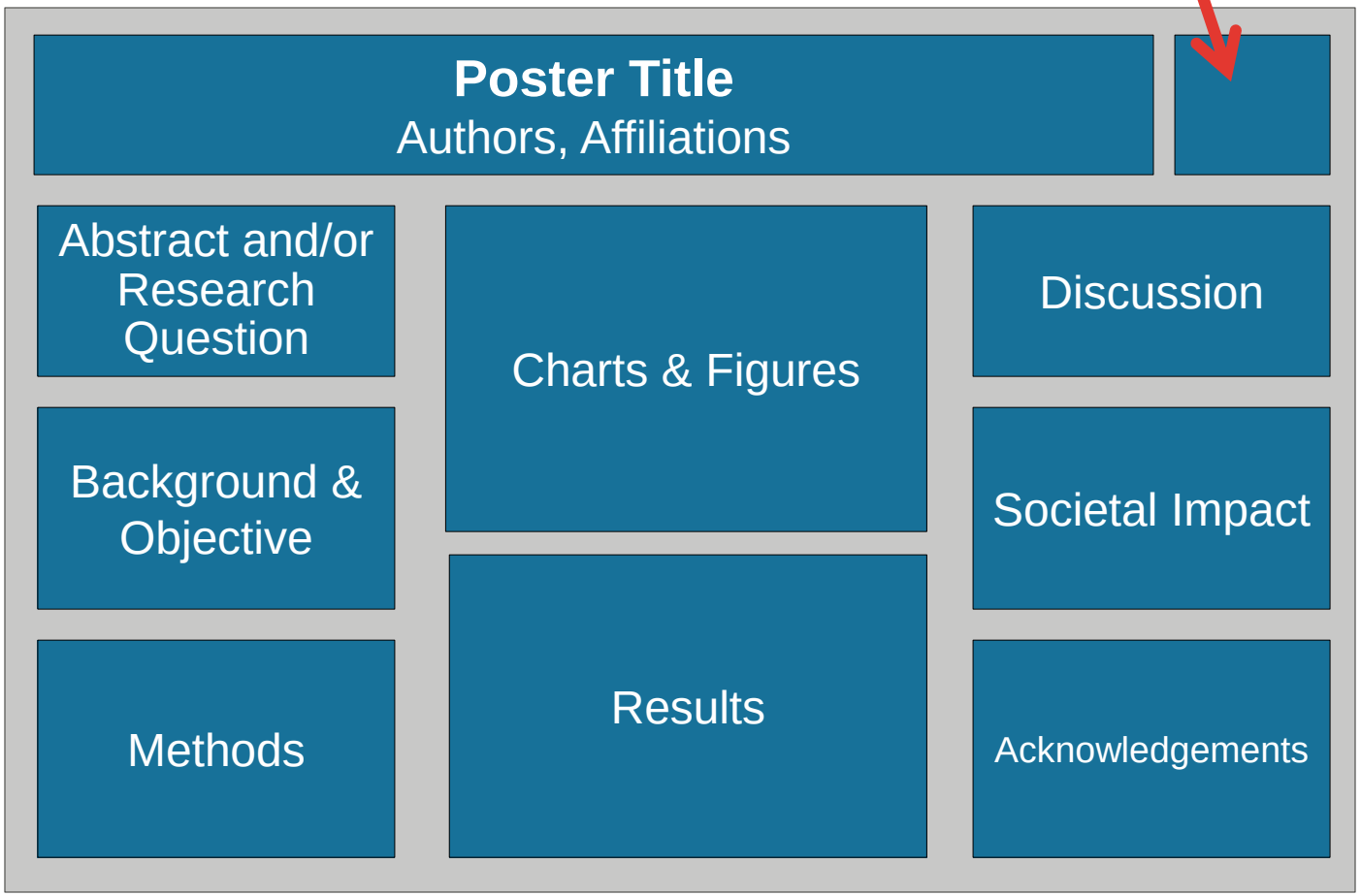
POSTER PREP: GENERAL POSTER FORMAT

Leave 4.5x5.5” blank space here for poster number that will be provided to you



2018 Research Day at the Capitol

POSTER PREP: A QUICK REFERENCE GUIDE

- Every poster should be custom made/tailored to the event you are preparing it for
- Your Research Day at the Capitol poster is NOT necessarily for a scientific crowd, it is for the general public and Legislators
- It is being judged by highly educated researchers from various fields

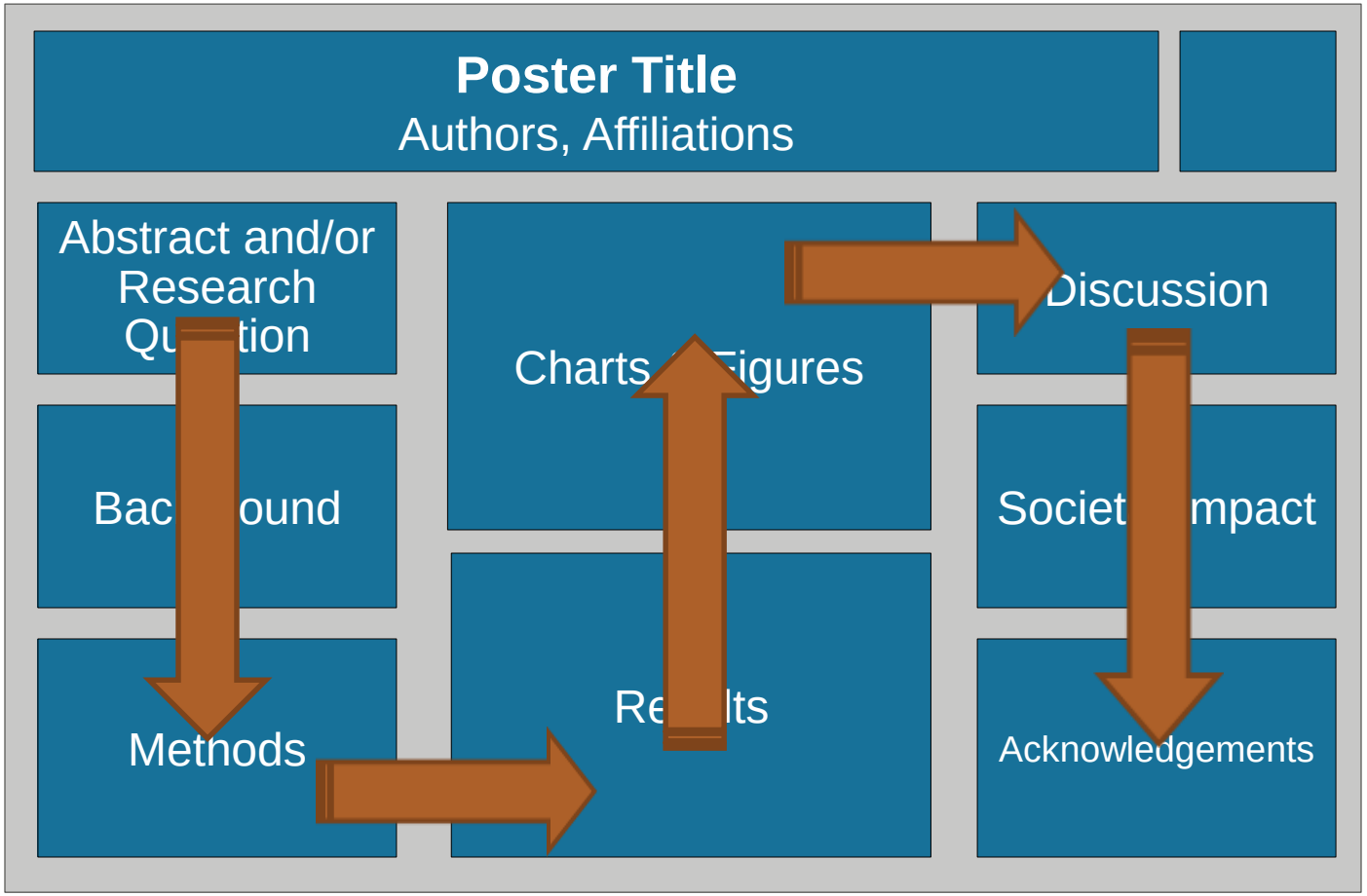
SO: Make sure your poster can be understood by the non-scientific community, but it must also succinctly express your scientific research and findings

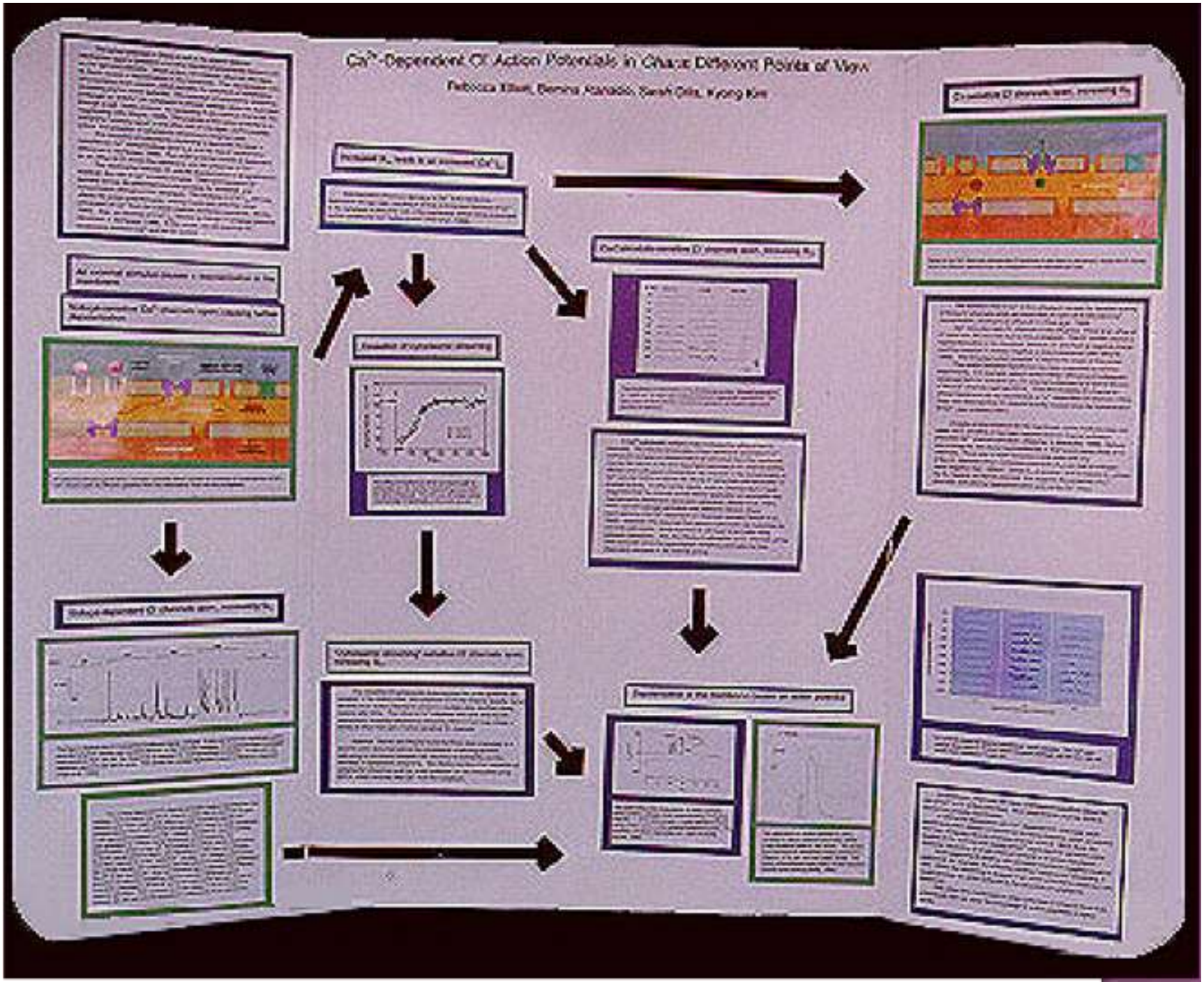
- Include all essential information; keep writing concise
- Avoid jargon

2018 Research Day at the Capitol

POSTER PREP: GENERAL POSTER FORMAT

Use logical flow between sections: top to bottom and left to right




Ca2+-Dependent AP Action Potentials in Chara: Different Points of View
Rebecca Elliot, Benisha Alahakoon, Sarah Orth, Kyong Kim

PMA induces growth inhibition and morphological changes in HT-1080 cells

Mary Katherine Randolph[1] and Zhizhuang Joe Zhao[2]

[1]Department of Chemistry, Oklahoma City Community College,

[2]Department of Pathology, University of Oklahoma Health Sciences Center

Abstract

Introduction: Ras oncogene activations are present in approximately 30% of human malignancies including colon, pancreas, thyroid and hematopoietic cancers. Our earlier studies reveal that oncogenic K-Ras-transformed cells are highly sensitive to inhibition by phorbol 12-myristate 13-acetate (PMA). In this study, we utilized a human fibrosarcoma cell line (HT-1080) with a mutated N-Ras allele to investigate further the effects of PMA on Ras-transformed cells.

Methods: The entire coding region of N-Ras was amplified from HT-1080 cell cDNA by PCR and sequenced. HT-1080 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C, 5% CO2, and humidified conditions in the presence or absence of PMA. Cell counts were obtained on a 2/10 mm hemocytometer and phase contrast microscope. Cell density and morphology were observed with Wright-Giemsa and immunofluorescence staining. Activation of Erk1/2 was assessed using Western blot analysis and immunofluorescence staining.

Results: Heterozygous N-RasQ61K mutation was found in HT-1080 cells. Cultures treated with a high dose of PMA (10uM) consistently showed a significant ($p<0.05$) decrease in cell number compared to the respective control culture. Results for HT-1080 cell cultures treated with a low dose of PMA (0.02uM) were less consistent and the decrease was not always significant ($p>0.05$). PMA-treated cells have a stretched appearance with prominent actin reorganization and appear differentiated.

Conclusions: PMA induces extensive cell growth inhibition and morphology changes in HT-1080 fibrosarcoma cells.

Introduction

The Ras proto-oncogenes encode small GTPases (N-Ras, H-Ras, and K-Ras) which act as molecular switches in regulating cellular proliferation, differentiation, and survival.[1] Non-transformed Ras proteins are only transiently active while oncogenic mutations create constitutively active Ras proteins.[1] This state results in constitutive activation of downstream effectors, including the Ras-Raf-Mek-Erk(p42/p44 MAPK) pathway, which is involved in cellular proliferation.[1] Oncogenic Ras mutations occur with a 30% frequency in cancers of the highest mortality.[1] We investigated the effect of PMA on the HT-1080 human fibrosarcoma cell line containing an endogenous mutated N-Ras allele. PMA mimics the endogenous activator diacylglycerol (DAG) to activate proteins across many different classes including novel and classical protein kinase C isozymes, protein kinase D isozymes, and Ras guanine nucleotide exchange factors which activate Ras proteins.[2] PMA is perhaps best known for its tumor promoting properties in the mouse skin carcinogenesis model. Prolonged topical application of PMA promotes skin tumors on mice previously exposed to a mutagenic carcinogen.[2] Our earlier studies, however, revealed that oncogenic K-Ras-transformed cells are highly sensitive to inhibition by PMA. Depending on the cell type, PMA is capable of promoting mitogenic responses or initiating growth arrest. Our goal was to investigate further the effects of PMA on N-Ras-transformed cells.

Acknowledgments

This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447 and by Oklahoma EPSCoR.

Results

Heterozygous N-RasQ61K mutation in HT-1080 Ce

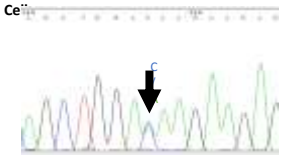


Figure 1. The entire coding region of the N-Ras gene was amplified from HT-1080 cell cDNA by PCR and sequenced. HT-1080 cells show heterozygosity for the N-Ras mutation with a single amino acid substitution at position 61, from a glutamine (Q) to a lysine (K).

Comparative Morphology

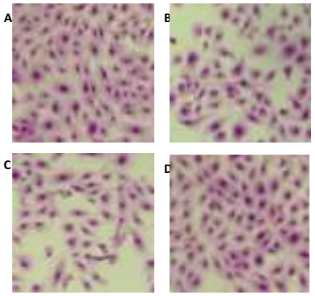


Figure 2. Morphology of investigated HT-1080 cells. (A) Control, (B) 10uM PMA, (C) 100uM PMA, and (D) 1000uM PMA. Cells in B-D are less dense, irregularly shaped and appear elongated. Wright-Giemsa stain, bright field, 100X.

Viable cell count

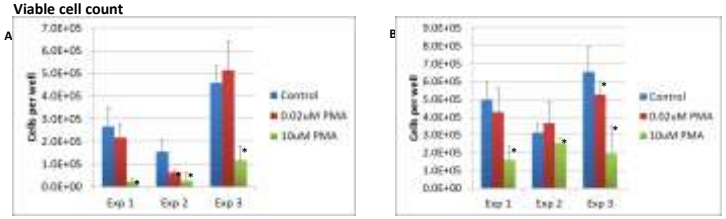


Figure 3. Experiments in set (A) were seeded with half the uL HT-1080 cells seeded in set (B). Four viable cell counts were taken per slide, error bars indicate standard deviation. *P<0.05 indicates PMA-treated cells are significantly different from the corresponding control cells.

Morphological changes, actin reorganization and Erk 1/2 activation by PMA in HT-1080 cells

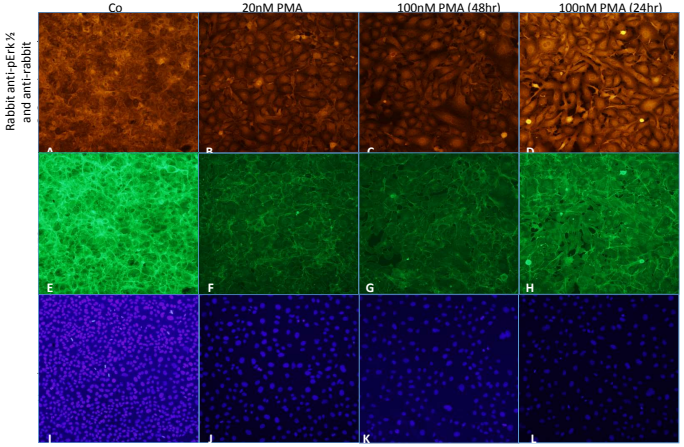


Figure 4. With the addition of PMA, A-D highlight increasing Erk 1/2 activation, E-H actin reorganization, and I-L increasing nucleus size and decreasing cell numbers. Brightly stained mitotic cells are visible in image I which are lacking in J-L after PMA treatment. Immunofluorescence microscopy, 200X.

Materials & Methods

Cell culture. Stock cultures of HT-1080 cells were maintained in DMEM supplemented with 10% FBS at 37°C, 5% CO_2, and humidified conditions. PMA dissolved in DMSO was added to cultures at a concentration of 0uM, 0.02uM, or 10uM. For each experiment to determine cell count, a high and low volume of HT-1080 cells were seeded from the stock culture; the low volume was always equivalent to half of the high volume. DMSO alone at the final concentration used in our experiments (<1%) is assumed to have no effect on cell growth. Cell numbers were determined on a 2/10 mm hemocytometer and phase contrast microscope (1X) after trypsinization in the presence of 0.05% EDTA.

Cell staining. HT-1080 cells were treated with 10uM, 100uM, 1000uM PMA or DMEM alone (control) for three days, fixed with methanol and stained with a Wright-Giemsa stain. For immunofluorescence microscopy, adherent HT-1080 cells were grown on glass coverslips. Cultures were treated with 20nM PMA for 48 hours, 100nM PMA for 48 hours, 100nM PMA for 24 hours or DMEM alone (control). Cells were fixed with 4% formaldehyde, permeabilized with 0.2% Triton X-100, and blocked with 50mM Tris-HCl to minimize nonspecific binding. Samples were then labeled with rabbit monoclonal antibody recognizing pERK 1/2 followed by goat anti-rabbit polyclonal secondary antibody Cy3-conjugated. Actin were stained with FITC-phalloidin dye and nuclei were stained with Hoechst 33258 dye.

Western blotting. Proteins were separated by SDS-PAGE, transferred to a PVDF membrane and blocked with 1% BSA. The membrane was probed with a rabbit monoclonal antibody recognizing pERK 1/2, washed and then probed with a goat anti-rabbit-HRP conjugated polyclonal secondary antibody. Chemiluminescent detection was performed and images were

Conclusions

- HT-1080 cells treated with PMA exhibited extensive growth inhibition as determined by cell counts three days after treatment. Growth inhibition was most effective at higher doses of PMA (10uM) with fewer HT-1080 cells seeded from the stock culture. The high dose of PMA (10uM) showed a significant ($p<0.05$) decrease in cell number in six out of six experiments, regardless of the initial concentration of HT-1080 cells seeded.
- In the presence of PMA, HT-1080 cells tend to be less dense in patches across the culture whereas the density is more uniform throughout control cultures.
- After PMA treatment, HT-1080 cells become stretched in appearance with prominent actin reorganization, more stress fibers are visible and the cells and nucleus appear larger. Single giant cells are also visible.
- Based on nuclear staining, the number of mitotic cells appear to decrease with the addition of PMA.
- Erk 1/2 becomes more active in the nucleus of PMA-treated cells.

Societal Impact

Both K-Ras and N-Ras transformed cells are sensitive to PMA treatment which may have implications for development of anti-cancer drugs targeting oncogenic RAS or its downstream effectors.

References

1. Takashima A, Faller D. Targeting the RAS oncogene. *Expert opinion on therapeutic targets.* 2013;17:507-531.
2. Griner E, Kazanietz M. Protein kinase C and other diacylglycerol effectors in cancer. *Nature.* 2007;7(4):281-294.

2018 Research Day at the Capitol

POSTER PREP: A QUICK REFERENCE GUIDE

Font Suggestions

- Use clear, simple fonts
  e.g. Times New Roman, Garamond, Arial, Century Gothic
- Title, 60-72 pt
- Authors & Institution, 38 pt
- Headings of boxes/sections , 42 pt
- Text of boxes/section, 26-32 pt
  (each column of text should have 11-12 words per line)
- Figure legends, 32 pt
- Acknowledgements, 26-32 pt
- Adjust font size as needed to fill your poster

2018 Research Day at the Capitol

POSTER PREP: A QUICK REFERENCE GUIDE

Graphics & Photos

- Use visual aids to tell your story (images, charts, diagrams, timelines)
- Minimal text to supplement the graphics
- Use titles, legends, consistent color (X and Y-axes should be labeled!)
- Be concise in your wording
- Text and graphics should be legible from three feet away
- Careful use of color (2-3 colors maximum)
- Photos must be min. 300 ppi
- Credit photos when appropriate

A Novel Polysulfide Synthesized Entirely From Waste and Its Use In Water Remediation

Austin M. Evans, Michael P. Crockett, Prof. Justin M. Chalker
The University of Tulsa Department of Chemistry and Biochemistry
Tulsa, Oklahoma, USA 74104

Abstract

Many functional materials today are prepared from non-renewable feedstocks. Addressing this issue, our research team has developed a novel polysulfide material synthesized entirely from the industrial waste products sulfur and limonene. This material is easy to synthesize on a large scale and is effective in removing toxic metals from water.

Background

Many chemical products are synthesized from non-renewable petroleum sources. Addressing this issue, our goal was to use abundant and renewable compounds as starting materials. Specifically, we reacted limonene and sulfur directly to form a polysulfide. 70,000 tons of limonene are produced as waste each year by the citrus industry. Sulfur is produced in the excess of 70,000,000 tons per year by the petroleum industry. Their wide availability has prompted exploration of these materials as chemical feedstocks.

Figure 1. Production of sulfur and Limonene

Because of the high sulfur content of our limonene-sulfur polysulfide, we hypothesized that it would bind to toxic metals and therefore be useful in removing toxic metals from water. This is particularly pertinent to Oklahoma because many of our waterways exhibit some form of toxic metal pollution.

Materials and Synthesis

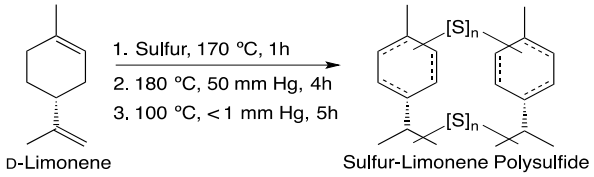


Figure 2. Reaction Scheme with proposed structure

Reaction Outline

1) Melt Sulfur (124 °C)
2) Heat to 170 °C (Radical Formation)
3) Add equal mass of limonene (b.p. = 176 °C)
4) Heat 1-5 hours at 170 °C
5) Process directly (mold, coat, etc)

Figure 3. Products of reaction

Reaction Features

1) No exogenous solvents or reagents
2) Completely atom economical
3) Operationally simple
4) Easily Scalable, 100 gram syntheses are routine

Water Remediation

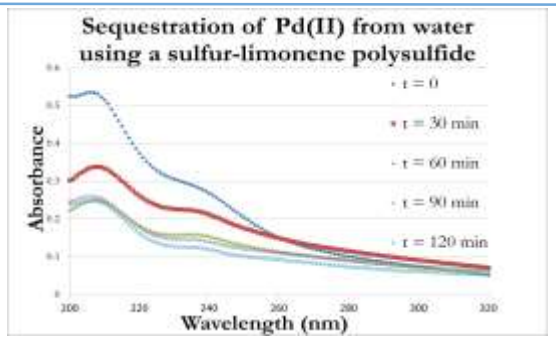
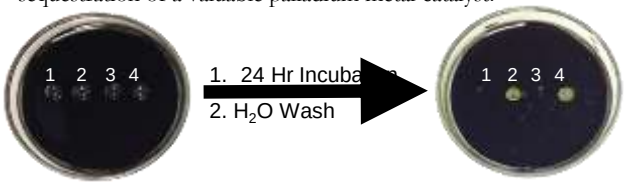


Figure 4. Palladium Catalyst Sequestration from Water

Using UV-Vis Spectroscopy, we monitored a time course of the sequestration of a valuable palladium metal catalyst.



1 = DI H_2O
2 = $HgCl_2$ in H_2O (2 mg/mL)
3 = Arkansas River water
4 = $HgCl_2$ spiked Arkansas River water (2 mg/mL)

Figure 5. Mercury sensing by a chromogenic response

Societal Impact

We have synthesized a novel polysulfide material entirely from industrial waste. The limonene-sulfur polysulfide is useful in removing metals from water, including mercury salts. We are currently investigating commercialization of this technology for on-site purification of natural waterways.

References

1. Chung, et al. *Nature Chemistry* **2013**, *5*, 518-524.
2. Polymers from Renewable Resources Gandini, A. *Macromolecules* **2008**
3. Crockett, M. P.; Evans, A. M.; Chalker, J. M. Unpublished
4. Sulfur-Limonene Polysulfide. Crockett, M. P.; Evans, A. M.; Chalker, J. M. Provisional patent filed Oct 24, 2014. No. 62068074.

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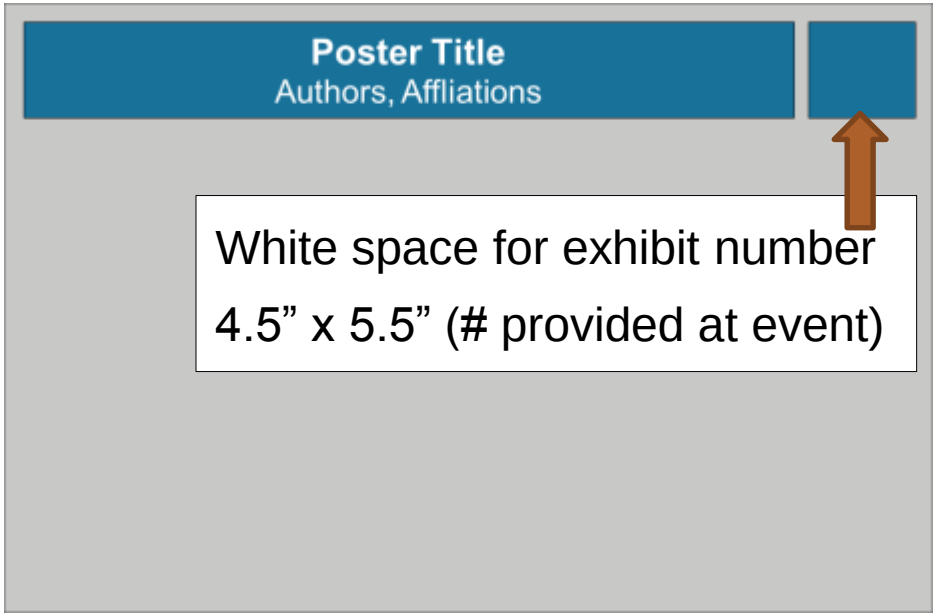
POSTER PREP: A QUICK REFERENCE GUIDE

- Title - Keep it simple & concise
- Authors – List all that were involved
- Institution – Campus <u>you are representing</u>



Obesity Control Using MicroRNAs

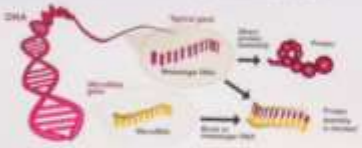
Lucille Redmond and Jin Seo
Dept. of Biology, Rogers State University, Claremore, OK 74017 USA

12

Background

- Since the rise of processed food and readily available, high-fat commodities, obesity has dramatically increased. Despite the correlation between poor diet and exercise, researchers have identified an innate biological element to obesity. This is a complex genetic disease not entirely understood, outside of the few genetic associations made thus far.

- MicroRNAs (miRNAs) are small, noncoding, RNAs 22 nucleotides in length. They bind to the 3' UTR untranslated region of their target mRNAs and repress protein production by destabilizing the mRNA, causing translational silencing. MiRNAs play a role in various cellular processes such as development, differentiation, and growth. This study explores the roles of miRNA on adiposity by observing effects of miRNA on triglyceride concentration in the *Drosophila melanogaster* (fruit fly), in hopes of identifying a connection between miRNA regulation and obesity.



Objectives of Study

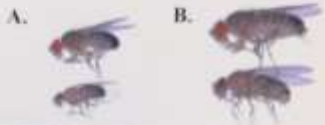
- Develop a better physiological understanding of obesity by identifying miRNAs that affect fat.
- Discover which miRNAs significantly positively or negatively influence triglyceride content

Societal Impact

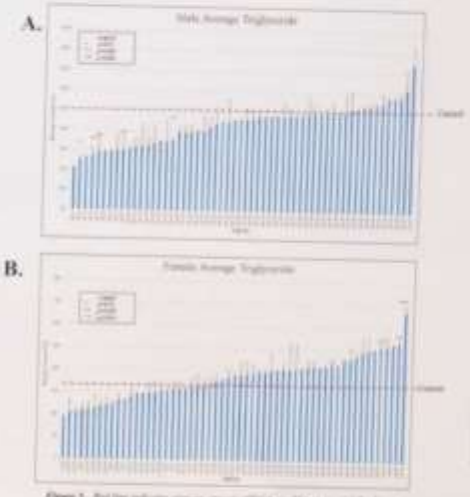
- Obesity can generate countless long-term health implications including heart disease, diabetes, and stroke.
- It affects society as a whole: not only those who require treatment, but taxpayers who subsidize healthcare for the obese.
- Oklahoma in particular has one of the highest obesity rates in the nation.
- This disease has become an economic and health crisis.

Methods

Mutant Fruit Flies



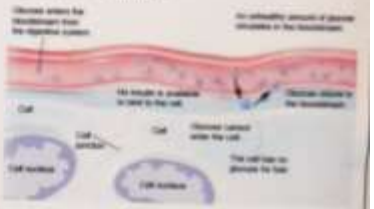
Results



Male v. Female

Discussion

- Insulin functions in maintaining energy homeostasis. It controls levels of circulating glucose and regulates storage of resources in the form of glycogen and fat.
- Low fat as a result of miRNA dysregulation of insulin signaling could mean that not enough glucose was transported into the cells, mimicking an insulin resistant state and causing cell to turn to the breakdown of fat for fuel.
- High fat is explainable by change in insulin pathway where too much glucose is being brought into the cell and excess stored as fat.
- Dysregulation of the insulin pathway could be a major cause of obesity. This disease may be modifiable by miRNA.



Conclusions

- Knockout of specific miRNAs caused nearly 30% increase and decrease in fat compared to control fly.
- Many of the miRNAs that cause a significant change in fat play essential roles in the insulin pathway.
- This study shows that genetically altering the insulin pathway could affect obesity.
- The cure to obesity and even diabetes may be within reach and as simple as manipulating miRNAs that regulate the insulin pathway.

Future Research

- Explore other screening mechanisms to further test miRNA correlation to obesity.

Acknowledgements

This work was supported by RSU ORLI Grant

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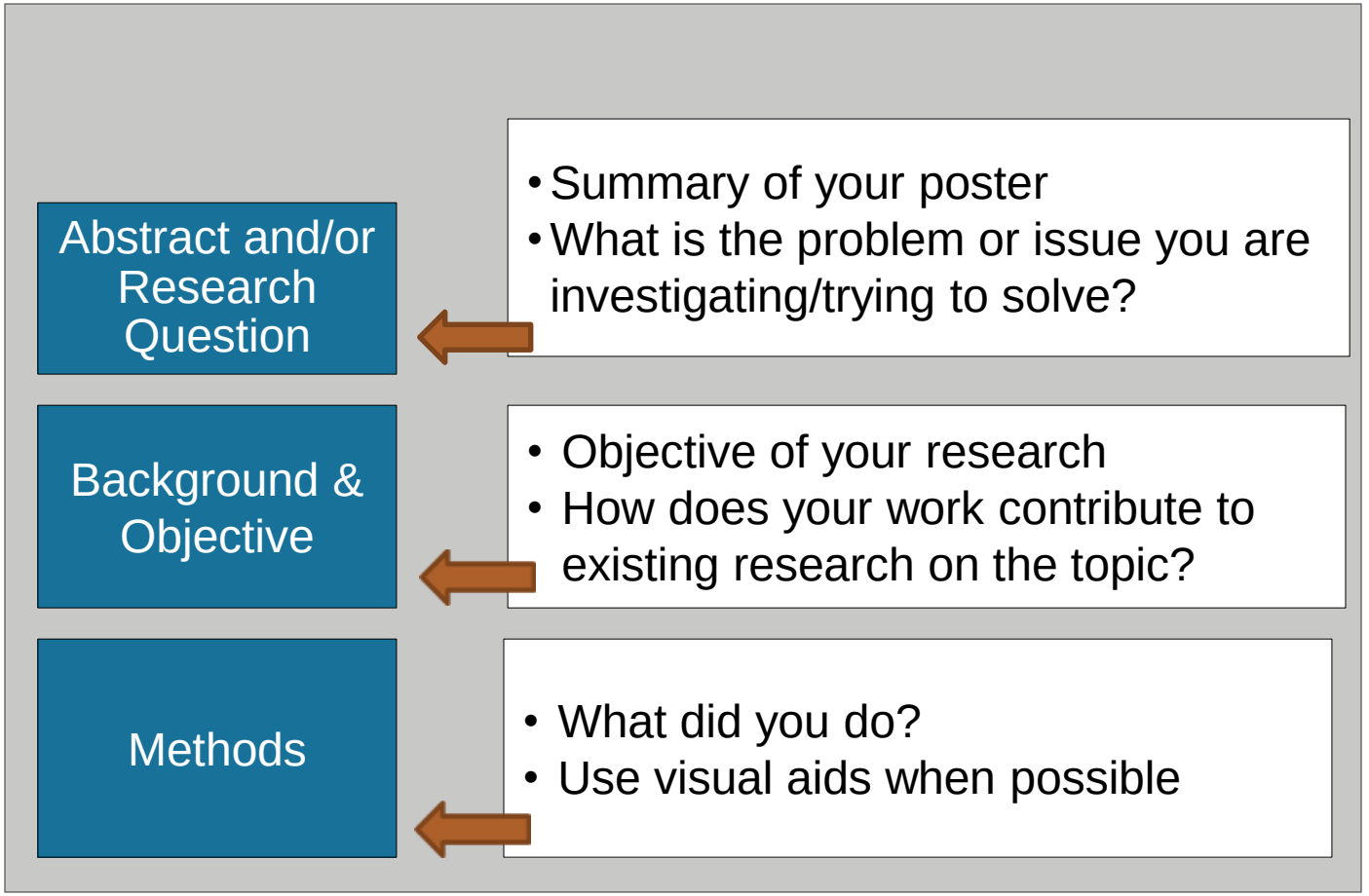

Impact of Wastewater Treatment Plant Effluent on Antibiotic Resistance in Aeromonads

Maegan Dallis, Samantha Henderson, Chrystal Moore, Kelley Dixon, Cindy Cisar

Department of Natural Sciences, Northeastern State University

ABSTRACT

Aeromonads, gram-negative bacteria belonging to the genus *Aeromonas*, are ubiquitous in freshwater ecosystems. Some species of aeromonads are opportunistic human pathogens while others have been linked to gastroenteritis in humans. Our objective in this study was to determine whether wastewater treatment plant (WWTP) effluent contributes to antibiotic resistance in aeromonads. Little is known about the impact of WWTP effluent on antibiotic resistance, one of the world's pressing public health problems. In November 2007, Tahlequah Creek water was analyzed for the presence of antibiotics, and bacteria were isolated from creek sediment. Samples were taken upstream and downstream of the Tahlequah wastewater treatment plant. No antibiotics were detected in the water samples taken upstream of the wastewater treatment plant, but four antibiotics were detected at subtherapeutic levels in the downstream water samples: azithromycin, ciprofloxacin, ofloxacin, and trimethoprim. Bacterial isolates from the sediments were identified at least to genus by sequencing their 16S ribosomal RNA genes. Forty-five aeromonad strains were isolated from sediment samples upstream of the WWTP, and twenty-eight aeromonad strains were isolated from sediment samples downstream of the WWTP. These isolates were tested for susceptibility to the antibiotics tetracycline, trimethoprim, and ofloxacin. Seven aeromonads were resistant to trimethoprim (3 upstream, 4 downstream), 6 aeromonads were resistant to tetracycline (2 upstream, 4 downstream), and 4 aeromonads were resistant to ofloxacin (all downstream). Ofloxacin is a second generation fluoroquinolone antibiotic that was approved by the Food and Drug Administration in 1990. We believe that this is the first report of ofloxacin resistance in aeromonads in the United States. Resistance to ofloxacin is of concern because fluoroquinolones are a relatively new class of broad spectrum antibiotics that can be used to treat bacterial infections when older antibiotics fail. We also determined that four of the downstream aeromonad strains exhibited multidrug resistance while none of the upstream strains did. Although the sample size is small, the data indicates a statistically significant increase in the incidence of antibiotic resistance in aeromonads exposed to effluent from the wastewater treatment plant. The Environmental Protection Agency does not currently regulate levels of antibiotics or antibiotic resistant bacteria in effluent released from wastewater treatment plants. Our data indicates that these emerging components of WWTP effluent may have a significant impact on endemic bacterial populations in these ecosystems.

INTRODUCTION

Bacterial diseases are controlled through the use of antibiotics. Not surprisingly, antibiotics have been reported as the second most commonly prescribed class of drugs in the United States. However, antibiotics are often overprescribed or taken inappropriately. Bacteria exposed to antibiotics are constantly evolving. Increased levels of antibiotics in water, the result of widespread use in humans and in agriculture, could lead to the development and spread of antibiotic resistance in bacteria. This would pose problems for infection control and increase healthcare costs. This project examines antibiotic resistance in aeromonads in a freshwater ecosystem that receives effluent from a wastewater treatment plant (WWTP), a potential source of both antibiotics and antibiotic resistant bacteria.

MATERIALS AND METHODS

Coliform test - water

Coliform test - sediment

Antibiotic susceptibility test

Table 1. Most Probable Number Data[*] for Total and Antibiotic Resistant Coliforms in Water Samples from November 2007

Date	Site[*]	Total coliforms	*E. coli*	Ampicillin resistant		Ofloxacin resistant		Tetracycline resistant	
				Total coliforms	*E. coli*	Total coliforms	*E. coli*	Total coliforms	*E. coli*
Nov 07	T	/	29.9 ± 0.1	2,359.0 ± 20.0	10.2 ± 4.3	4.2 ± 1.1	3.6 ± 0.5	1,674.7 ± 668.5	23.6 ± 2.7
	T'	3,986.7 ± 440.1	273.3 ± 124.7	1,689.8 ± 245.3	84.8 ± 10.0	10.6 ± 2.9	5.8 ± 0.6	341.6 ± 11.1	65.7 ± 12.9

MPNs were determined in water samples using the Colisure® quantitray system (IDEXX Laboratories). Values are MPN per 100 ml water ± SEM.

[*] T is water from Tahlequah Creek sampled approximately 0.5 miles upstream of the WWTP. T' is the effluent from the Tahlequah WWTP.

No data available.

[*]Tahlequah WWTP was undergoing repairs on the date the effluent was sampled.

Table 2. Aeromonads Isolated in November 2007

Location	Number	Identification[*]
Upstream sediment	45	*Aeromonas* spp. (23) *Aeromonas hydrophila* (22)
Downstream sediment	28	*Aeromonas* spp. (5) *A. hydrophila* (23)
WWTP effluent	1	*A. hydrophila* (1)

[*]Identification is based on 16S rDNA sequences. Numbers in parentheses indicate number of isolates.

Table 3. Antibiotic Susceptibility of Aeromonads Isolated in November 2007

Location	Antibiotic	Number	Susceptible / Resistant	Multidrug Resistance
Upstream sediment	Ofloxacin	45	(45 of 45) susceptible — 100% none resistant — 0%	none
	Tetracycline	45	(43 of 45) susceptible — 95.6% (2 of 45) resistant — 4.4%	
	Trimethoprim	45	(44 of 45) susceptible — 97.8% (1 of 45) resistant — 2.2%	
Downstream sediment	Ofloxacin	28	(24 of 28) susceptible — 85.7% (4 of 28) resistant — 14.3%	2 resistant to ofloxacin and trimethoprim 1 resistant to tetracycline and trimethoprim 1 resistant to tetracycline, trimethoprim and ofloxacin
	Tetracycline	28	(24 of 28) susceptible — 85.7% (4 of 28) resistant — 14.3%	
	Trimethoprim	28*	(21 of 27) susceptible — 77.8% (6 of 27) resistant — 22.2%	

[*] One strain was used to determine susceptibility to antibiotics based on Clinical Laboratory Standards Institute guidelines.

[*]No criteria for tetracycline.

SOCIETAL IMPACT

Antibiotic resistant pathogens are a serious threat to human health. We have determined that wastewater treatment plant effluent, a source of antibiotics and antibiotic resistant bacteria, can contribute to antibiotic resistance in downstream bacteria populations. development of best practices to reduce the amounts of antibiotics and antibiotic resistant bacteria released into the environment may help in preventing the spread of antibiotic resistance in bacteria.

RESULTS

In November 2007 four antibiotics were present in Tahlequah Creek water samples collected downstream of the WWTP: azithromycin (0.002 µg/L), ciprofloxacin (0.006 µg/L), ofloxacin (0.009 µg/L), and trimethoprim (0.024 µg/L). No antibiotics were detected upstream of the WWTP. In addition, antibiotic resistant bacteria were present in Tahlequah Creek water and in WWTP effluent (Table 1). Many bacteria collected from Tahlequah Creek sediments in November 2007 were identified as aeromonads (Table 2). Forty-five aeromonad strains were isolated from sediment samples upstream of the WWTP and 28 aeromonad strains were isolated from sediment samples downstream of the WWTP. Of these, 7 strains were resistant to trimethoprim, 6 strains were resistant to tetracycline and 4 strains were resistant to ofloxacin. Several of the downstream aeromonad isolates were resistant to more than one antibiotic and one downstream aeromonad was resistant to two additional antibiotics (Table 3). Numbers of antibiotic resistant aeromonads were compared using a chi-square contingency test with Yates correction for small sample size. There were significantly more antibiotic resistant aeromonads present in sediments downstream of the WWTP than upstream of the WWTP in November 2007 ($P = 0.011$).

DISCUSSION

- Antibiotics and antibiotic resistant bacteria were both present in this freshwater ecosystem. However, antibiotic resistant aeromonads were more likely to be found downstream than upstream of the WWTP suggesting that WWTP effluent contributes to antibiotic resistance in aeromonads.
- Roughly equal numbers of bacteria were isolated from sediments upstream and downstream of the WWTP, but the ratio of aeromonads to other bacteria was lower in the downstream bacterial population. Therefore, although more likely to be resistant to antibiotics the downstream aeromonad population appeared to be negatively impacted by the WWTP effluent.
- Four aeromonad isolates from downstream of the WWTP were resistant to ofloxacin. To our knowledge, this is the first report of ofloxacin resistance in aeromonads in the United States.

We are currently analyzing the genes responsible for antibiotic resistance in the aeromonad strains. Ultimately, we plan to quantify the rate of occurrence of horizontal transfer of antibiotic resistance in bacteria in the environment, identify the transfer mechanism(s) involved, and assess the impact of environmental reservoirs of antibiotic resistance on human pathogens and disease.

ACKNOWLEDGEMENTS

Funding was provided by the Oklahoma Center for the Advancement of Science and Technology, OSRHE award HR07-124, and by NIH/NCRR grant P20RR016478-04.

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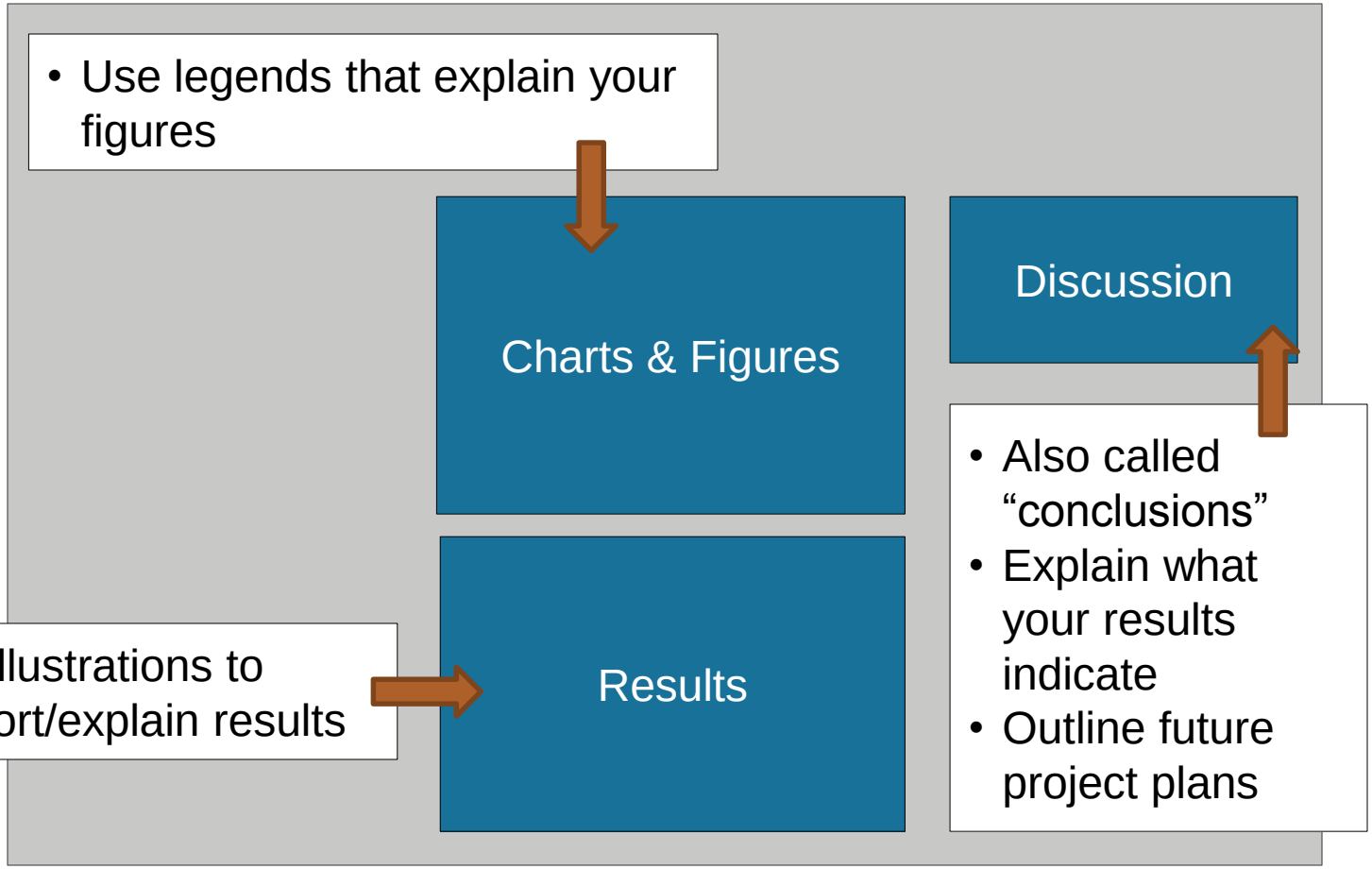

A Novel Assay to Predict Cancer Resistance to Cisplatin

Lacy Brame[1], Vengatesh Ganapathy[1], Ilangovan Ramachandran[1], Lurdes Queimado[1-5]

Departments of [1]Otorhinolaryngology, [2]Cell Biology and [3]Pediatrics; [4]The Oklahoma Tobacco Research Center and [5]The Peggy and Charles Stephenson Cancer Center, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA.

Introduction

- Cisplatin is widely used as chemotherapy drug that induces DNA damage and ultimately triggers apoptosis. However, therapeutic resistance and tumor relapse remains a significant clinical problem.
- Recently, our laboratory developed an assay (Fig. 1) called primer anchored DNA damage detection assay (PADDA) that screens genomic areas for DNA damage[1]. PADDA has been shown to detect a dose-dependent increase in DNA damage caused by genotoxic agent (Fig. 2).
- We hypothesized that PADDA will discriminate the ability of cancer cells to repair damage induced by cisplatin, and therefore predict cancer resistance to cisplatin.

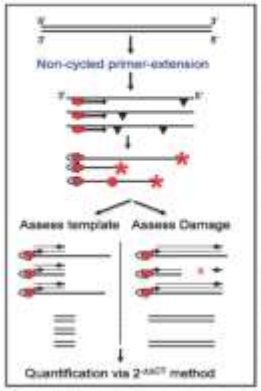

Figure 1. Diagram of PADDA. A single strand-specific non-cycled primer extension performed with a 5'-biotin-tagged primer and Vent exo- DNA polymerase identifies damaged nucleotides (inverted triangles), and generates a pool of highly specific biotin-tagged extended products, each of them derived from one strand of a single DNA molecule. Each extended product has a stop, which represents replicative arrest by a damaged nucleotide or nick. Some extended products will contain misincorporations that represent polymerase lesion by-pass with misincorporation. After several purification steps, the strand-specific, biotin-bound extended products can be used for damage quantification on a high throughput setting q-PADDA).

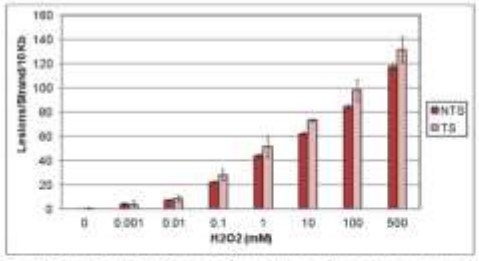

Figure 2. Quantification of induced DNA damage after *in vitro* exposure to a dose escalation of H_2O_2. Strand-specific DNA damage was quantified by q-PADDA. Lesion frequency was estimated via Poisson equation. NTS, non-transcribed strand; TS, transcribed strand; Data represents Mean ± S.E.M

Aim

To define the levels of DNA damage induced at p53 nucleotides by cisplatin treatment and to measure the ability of cancer cells to repair damage induced by cisplatin.

Materials & Methods

PADDA was used on a high-throughput setting to quantify DNA damage in human oral cancer cells (SCC-1) exposed to different doses of cisplatin. Cell viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Data was analyzed by Student's *t*-test.

Results

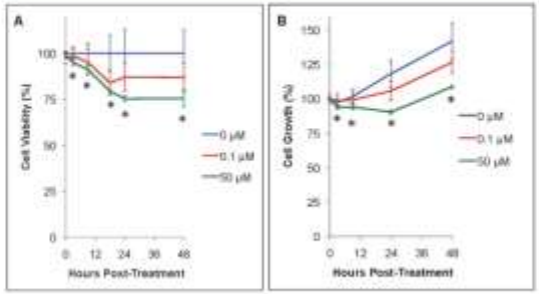

Figure 3. Cell viability assay and cell growth. SCC-1 cells were treated with 0 µM, 0.1 µM, 50 µM concentrations of cisplatin and allowed to repair damage for 0, 3, 9, 18, 24 & 48 hour time intervals. Cell viability (A) and cell growth (B) were determined by MTT assay. Data shown as Mean ± S.D. * $p<0.01$.

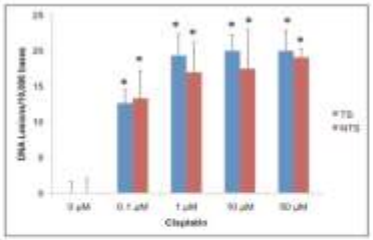

Figure 4. DNA damage measured by q-PADDA in SCC-1 cells exposed to cisplatin for 3 hours. Damage was quantified by q-PADDA in both transcribed (TS) and non-transcribed (NTS) strands. Data shown as Mean ± S.E.M. * $p<0.01$.

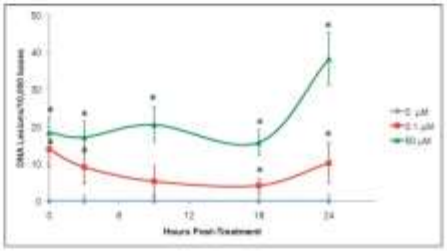

Figure 5. DNA damage measured by q-PADDA in SCC-1 cells after exposure to cisplatin. SCC-1 cells were treated with 0 µM, 0.1 µM, 50 µM concentrations of cisplatin and allowed to repair damage for 0, 3, 9, 18 & 24 hour time intervals. Data shown as Mean ± S.E.M. * $p<0.01$.

Conclusion & Societal Impact

- PADDA was able to quantify DNA damage and repair after cisplatin treatment. This information will allow us to determine if resistance to cisplatin is due to effective damage removal or to damage tolerance. This data would facilitate the development of strategies targeting the mechanism of drug resistance.
- This observation has **significant clinical importance** as it can be used to predict treatment response and direct treatment selection in cancer patients.

Future Directions

- This assay has potential to elucidate the differential efficacy of cisplatin as a chemotherapy drug and act as a preliminary screening method to determine differential cisplatin resistance.
- This project can be extended to determine the genotoxicity and resistance of cisplatin in other head and neck cancer cell lines.
- PADDA can be used to determine if patients will respond or become resistant to not only platinum-based chemotherapy treatments, but also to other treatments that induce DNA damage.

Acknowledgement

Funding was provided by the Oklahoma Tobacco Research Center and the Oklahoma Center for the Advancement of Science and Technology. Dr. Queimado holds a Presbyterian Health Foundation Endowed Chair in Otorhinolaryngology.

References

[1]Reis AM, Mills WK, Ramachandran I, Friedberg EC, Thompson D and Queimado L. Targeted detection of *in vivo* endogenous DNA base damage reveals preferential base excision repair in the transcribed strand. Nucleic Acids Res. 40(1): 206-219, 2012.

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POSTER PREP: GENERAL POSTER FORMAT

- DO NOT OVERLOOK THIS SECTION!!!
- Arguably one of the most important
- 2-3 concise sentences
- Explain the social benefits of your research in layman's terms

→ Societal Impact

Acknowledge your:

- Funding source(s)
- Collaborators (big and small)
- Journal articles used as references
- EPSCoR

→ Acknowledgements

Development in Potential Anti-HIV & Antimetastatic Drugs: C -Symmetric Tris-Linked Bridged Tetraazamacrocycles as Potential CXCR4 Antagonists

Courtney D. Garcia[1], B. N. Shockey[1], B. Gridley[2], S. J. Archibald[2], Dominique Schols[3], T. J. Hubin[1]

1. Department of Chemistry, Southwestern Oklahoma State University, 100 Campus Drive, Weatherford, OK 73096 USA
2. Department of Chemistry, University of Hull, Cottingham Road, Hull, HU6 7RX, UK
3. University of Leuven, Belgium.

1. Societal Impact:

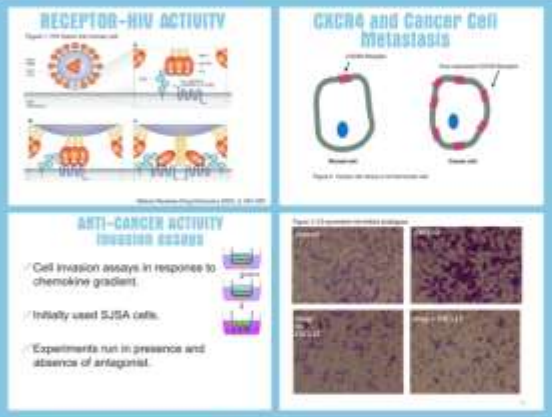
CXCR4 chemokine receptors are found on the surface of immune, and other, cells, and together with the specific natural ligand, CXCL12,have been revealed to play a role in a number of disease states. CXCR4 expression has also been reported in at least 23 different cancers. Target organs for breast metastases such as liver, lung, and bone have high levels of CXCL12. Due to the wide-ranging potential biomedical applications that might result, our aim is to develop new antagonists for the CXCR4 co-receptor.

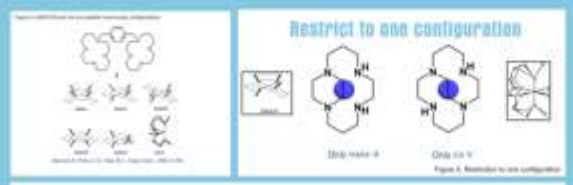
2. Objectives:

Our objectives were to synthesize C3-symmetric tris-linked analogues of our most effective bis-tetraazamacrocycle metal complexes and to characterize their chemical and physical properties in preparation for determining if the added macrocycle enhances their antagonism of CXCR4.

3.Methods:

Synthetic routes extending our bis-linked ligand syntheses to use the C 3-symmetric linker 1,3,5-tris(bromomethyl)benzene were developed. Copper(II), nickel(II), cobalt(II), and zinc(II) complexes were made using our previous methods. Electrospray mass spectra, UV-Visible spectra, cyclic voltammograms, magnetic moments, X-Ray crystal structures, and ^{1}H and ^{13}C NMR spectra were collected to characterize the complexes.





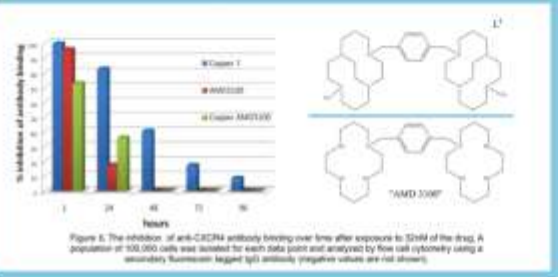
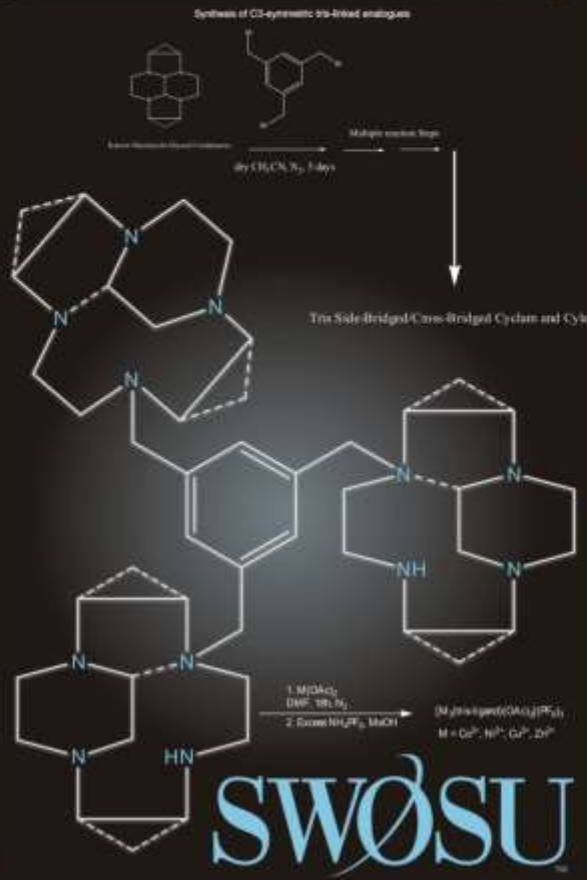


Figure 6. The inhibition of anti-CXCR4 antibody binding over time after exposure to 32nM of the drug. A population of 100,000 cells was isolated for each data point and analyzed by flow cell cytometry using a secondary fluorescein tagged IgG antibody (negative values are not shown).



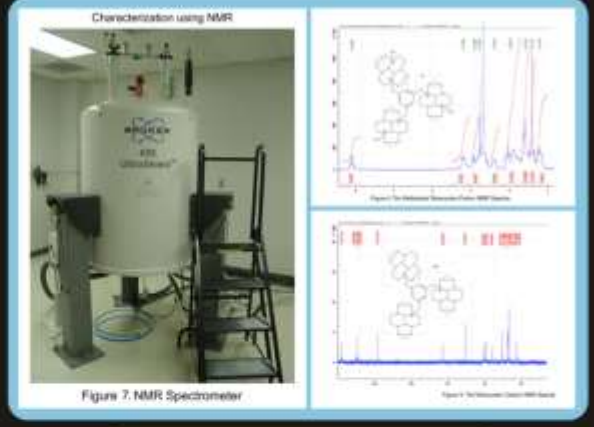
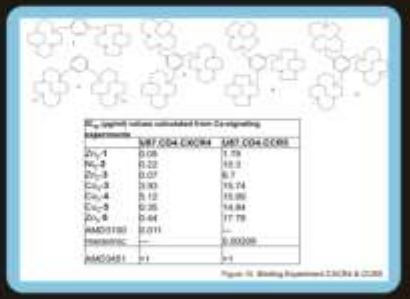


Figure 7. NMR Spectrometer



	MB7.CD4.CXCR4	MB7.CD4.CCR5
Zn_2-1	0.08	1.79
Ni_2-2	0.22	10.3
Zn_2-3	0.07	8.7
Cu_2-3	0.93	15.74
Cu_2-4	5.12	15.85
Cu_2-5	0.35	14.84
Zn_2-6	0.44	17.78
AMD3100	0.011	>>
Haranozic	>>	0.00206
AMD3451	>1	>1

4. Results:

The ligand syntheses of the side-bridged and cross-bridged C_3-symmetric ligands proceeded similarly to the previously developed bis-ligand routes. Complexation with the desired metal ions proceeded as expected. Characterization of the metal complexes resulted in publishable quality purity in each step of synthesis. Experiments investigating the Calcium release have shown that the C3-symmetric compounds are highly potent as CXCR4 antagonists, just as in the bis-linked compounds. An unexpected benefit of tris linking is CCR5 binding. CCR5 is another important chemokine receptor.

5. Conclusions:

C3-symmetric tris-linked bridged tetraazamacrocycles are easily produced, using an appropriate linker and following synthetic methods adapted from the bis-linked analogues. Metal ion complexation proceeds smoothly following known procedures. Calcium ion release is observed when the natural ligand for CXCR4, CXCL12, binds. Preventing Calcium release is evidence of strong antagonism by the potential drug molecule. Also, several of the C3-symmetric compounds have demonstrated excellent antagonism of a related chemokine receptor, CCR5, as well. This exciting result may lead to a new class of dual chemokine receptor antagonists.

6. Future plans:

Experimental data on the specific disease states of HIV infection and cancer with the resulting complexes will inform our understanding of the requirements for producing even more efficient CXCR4 antagonists of this class.

Acknowlegements: Funding was provided by Research Corporation (CC6505); the Oklahoma State Regents for Higher Education; and NIH Grant P20 RR016478 from the INBRE Program of the National Resources.

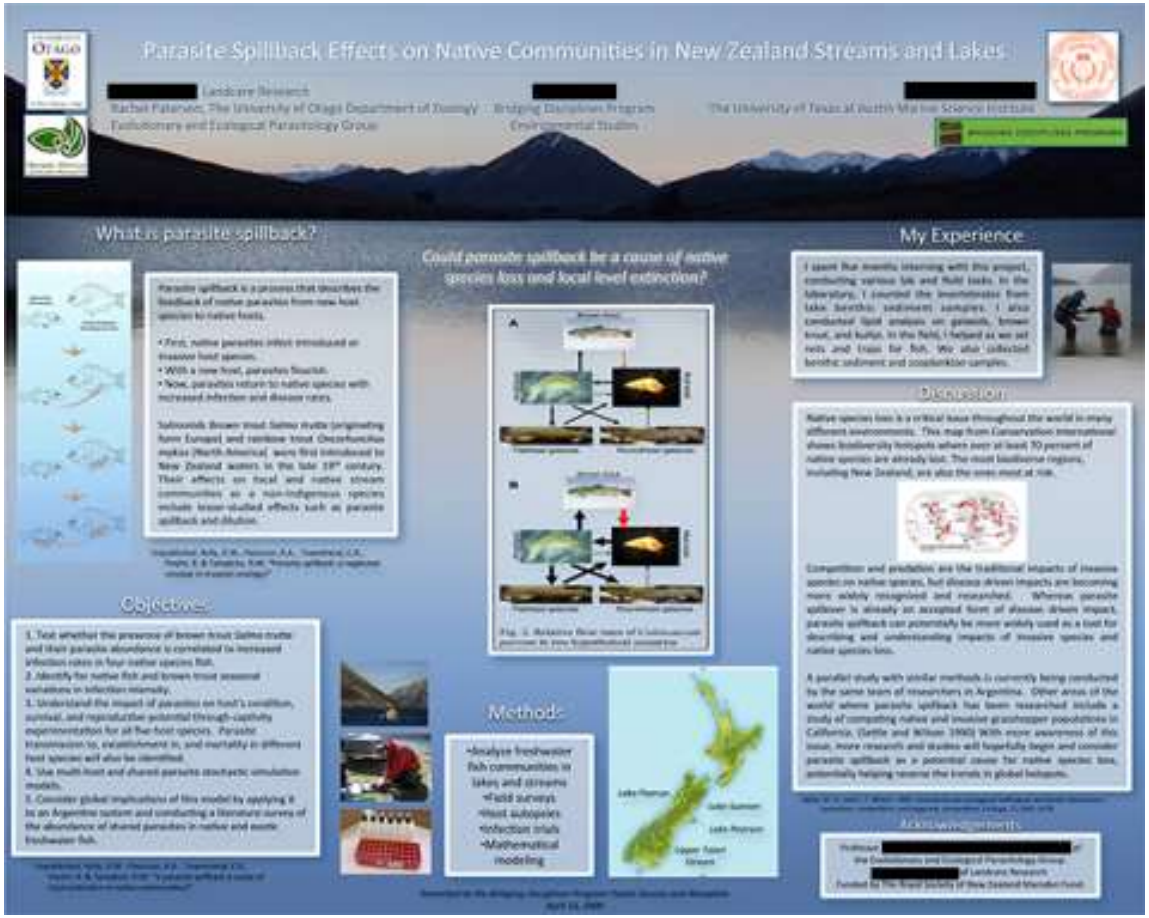
Poster Examples: "Do's" and "Don'ts"

Strengths:

- Logical order
- Various visual aid types
- Acknowledgements

Weaknesses:

- Sections & images not aligned
- Distracting background
- Too many visual components



Credit: University of Texas at Austin, https://ugs.utexas.edu/our/poster/samples

An example of why you should NOT use a photo or graphic as your poster background.

Text is impossible to read and potential observers would be too distracted by the image to sort through the information anyway.

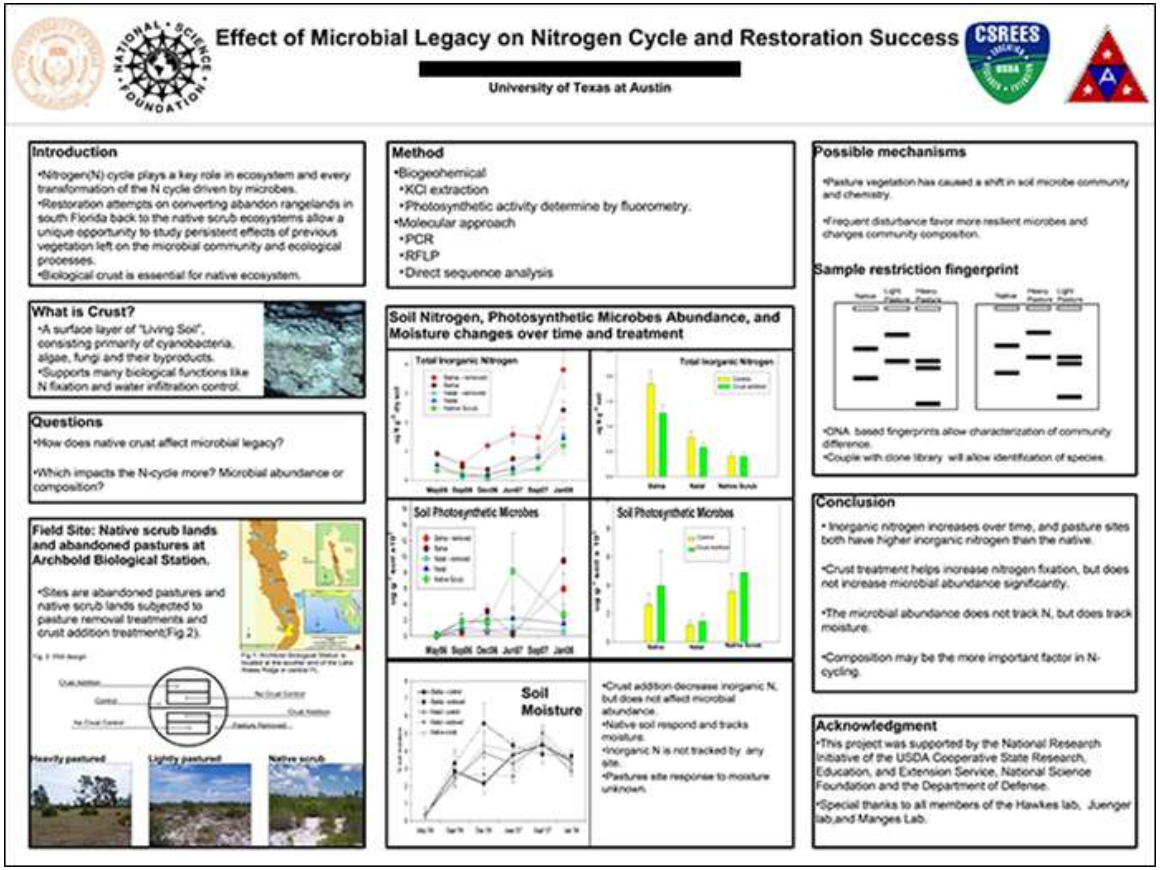
Poster Examples: "Do's" and "Don'ts"

Strengths:

- Clearly defined research questions
- Effective use of visual aids
- Clear organizational structure
- Bullets break up text

Weaknesses:

- Technical language & undefined acronyms (limits audience)
- Narrow margins within text boxes
- Too many thick borders around boxes
- Uses incorrect logo for the institution



Credit: University of Texas at Austin, https://ugs.utexas.edu/our/poster/samples

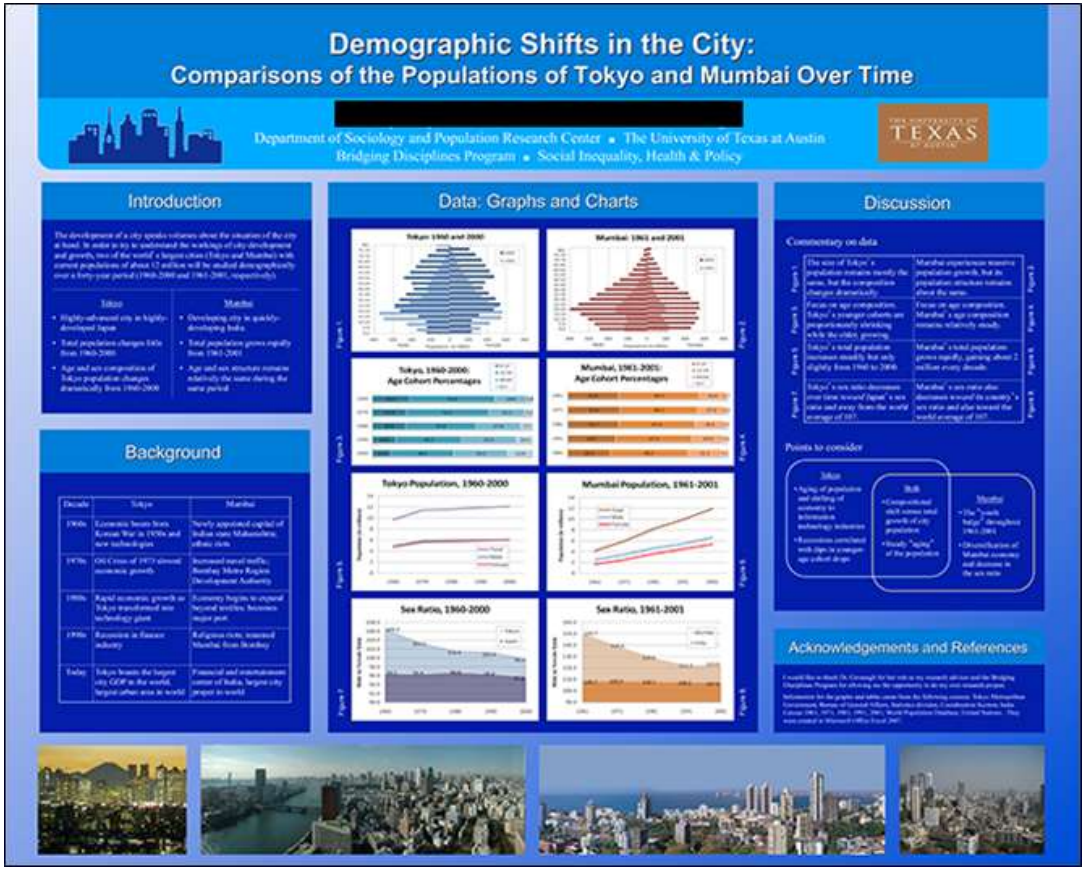
Poster Examples: "Do's" and "Don'ts"

Strengths:

- Venn diagram in discussion
- Consistent graphics
- Multiple types of visual aids

Weaknesses:

- Light text on dark background
- Color backgrounds should be avoided, especially dark ones
- Unlabeled, non-credited photos



Credit: University of Texas at Austin, https://ugs.utexas.edu/our/poster/samples

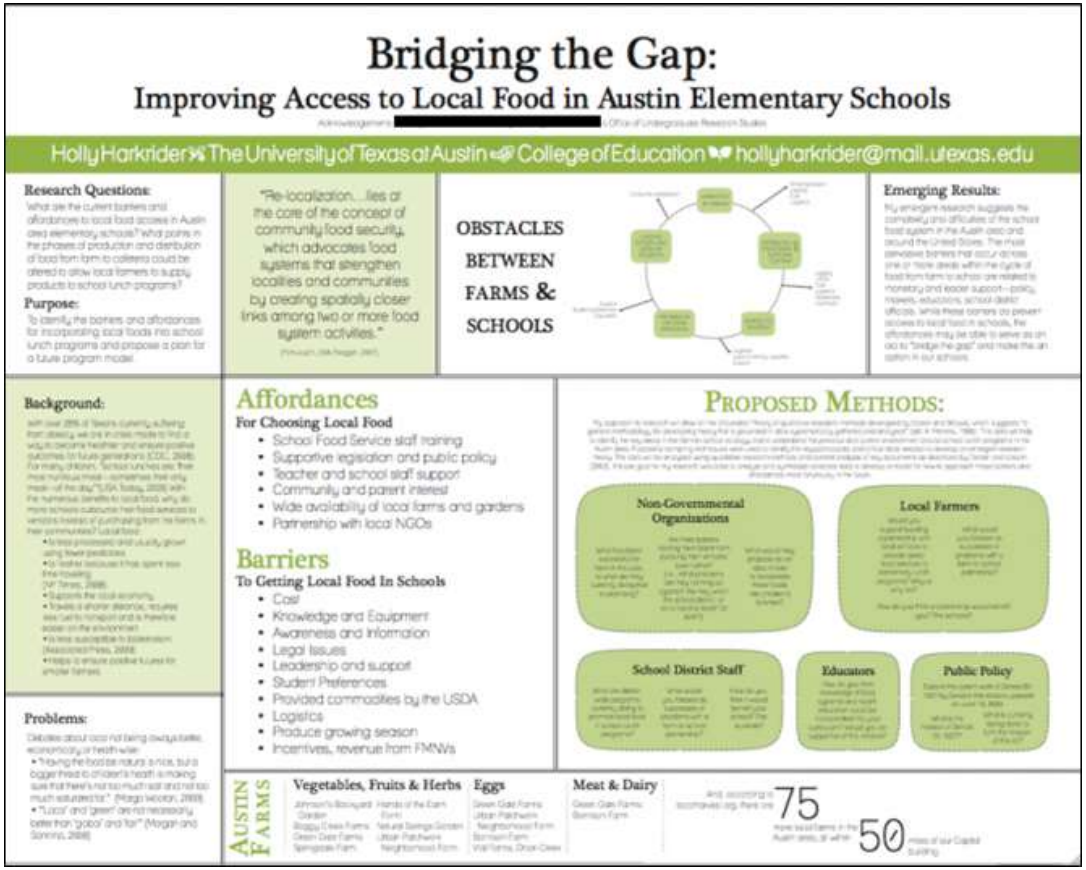
Poster Examples: "Do's" and "Don'ts"

Strengths:

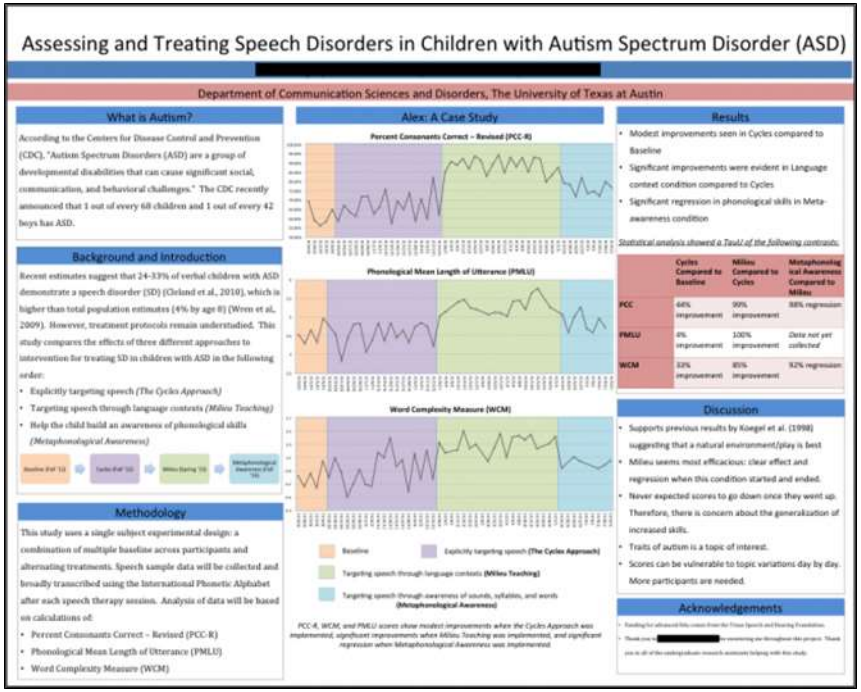
- Easy to read
- Clearly defined research question
- Use of white space
- Simple color scheme
- Use of shapes, figures, bullets to break up text
- Compelling title (and title font size)
- Clean visual impression

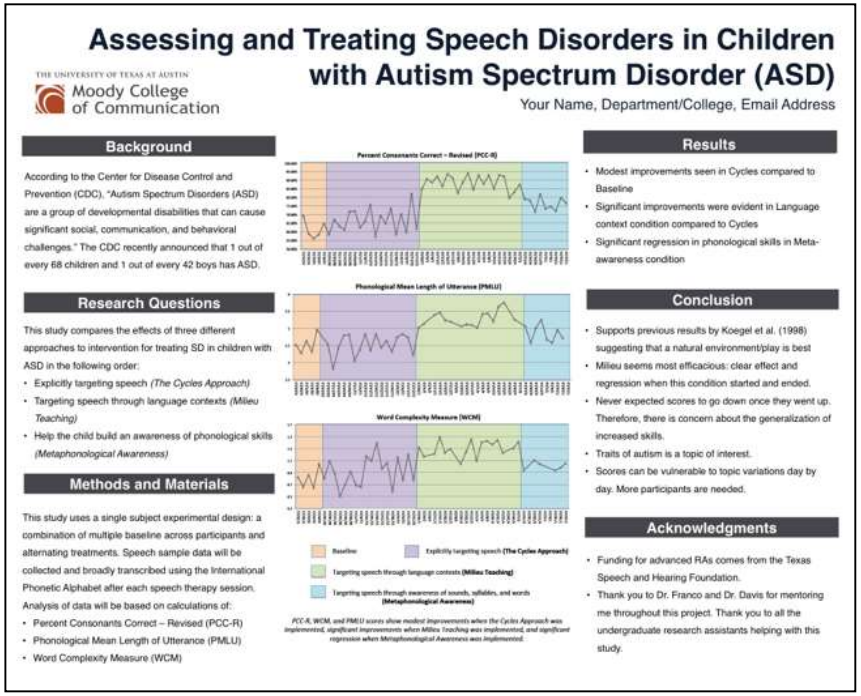
Weaknesses:

- Many sections without a clear flow between them
- Lacks acknowledgements



Poster Examples: Before/After





Credit: University of Texas at Austin, https://ugs.utexas.edu/our/poster/templates

An Online Poster Prep Resource
https://ugs.utexas.edu/our/poster

The University of Texas at Austin's online Poster Guide is a great resource, providing thorough and easy-to-understand scientific poster design tips and instruction.

- Guide to Creating Research Posters
- Poster Samples: What to do and what not to do
- Poster Content Development
- Organizing Poster Content
- Poster Design Elements and Guidelines
- Review Your Poster
- Printing Your Poster
- Presenting Your Poster
- Talking About Your Poster

2018 Research Day at the Capitol

ONLINE REGISTRATION REQUIRED (BY MARCH 12)

All student researchers & anyone who will be attending Research Day at the Capitol activities in support of the student researcher must register online at: http://www.okepscor.org/calendar/2018-03-27

- Please advise parents, friends, family, faculty advisors, etc. to register online (or you may register online for them)
- Why? This event is funded through a grant from the National Science Foundation. NSF requires participants information to continue funding for the event.
- Registration deadline: March 12

2018 Research Day at the Capitol

FINAL THOUGHTS—THINGS TO REMEMBER

You were chosen for a reason!

- Be enthusiastic, friendly, and SMILE
- Be ready and mentally prepared—practice!
- Emphasize your societal impact
- Dress professionally and be punctual
- Know your Legislators and engage them
- Judges are looking for someone who has the whole package!

2018 Research Day at the Capitol

INSIGHT FROM A PAST WINNER

Madison Duckwall, Grand Prize Winner 2017

Southwestern Oklahoma State University

Poster Topic: Synthetic Biology

REAL WORLD SYNTHETIC BIOLOGY:

Production of Arsenic Biosensors and Aspirin Producing Bacteria

Authors: Madison J. Duckwall and Lori Gwyn Southwestern Oklahoma State University

Abstract: Synthetic biology is an emerging field that applies engineering principles to biological systems to solve problems. Biobricks, a molecular toolbox of genes with varied functions, have compatible enzyme sites that function as cut and paste locations so that one can "build" bacteria to accomplish a specified purpose. Arsenic is toxic when present in high quantities (>10 ppb) in water. Medications are complicated and occasionally dangerous to synthesize. Utilizing synthetic biology to modify bacteria to become sensors and synthesizers will address these problems. Arsenic biosensors capable of detecting micro quantities of arsenic in drinking water was goal one. Three parts were used to build an arsenic sensor: an arsenic promoter, a red reporter, and destination. The promoter is the on switch activated in the presence of micro to nano arsenic concentrations, which then signals the reporter to make the bacteria grow red/pink. The destination is a place to seal the promoter and reporter together in a predicted manner as well as provide antibiotic resistance to further select only desired bacteria. BioBricks were also used to develop aspirin synthesizing bacteria. Proposed parts for this purpose include an isochorismate synthase and acetyltransferase. Experiments are underway to test the activity of proteins produced by these parts. It is expected that the bacteria will utilize their own metabolites (salicylic acid) to make aspirin to be secreted into its surroundings. This project is relevant as a proof of concept that drugs can be synthesized easily with bacteria. We have successfully made an arsenic biosensor and plan to make it more sensitive and selective. Preliminary experiments of the aspirin project show that the E. coli can grow in ≤0.1% salicylic acid and up to 0.1% acetic acid. This indicates that the completed aspirin synthesizer should not be inhibited by the acidic environment it will create.

Arsenic Biosensor

Intro:

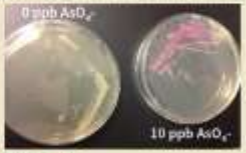
The EPA accepted level of arsenic in drinking water is 10 parts per billion (ppb). This is a worldwide problem as well as local.

In Weatherford, OK approximately $759,700 were spent as one method of decreasing arsenic concentration in the drinking water by 1ppb.

In Terai, Nepal 26% of the wells show arsenic concentrations up to 50 ppb.

Arsenic biosensors can be useful in determining if there are dangerous levels of arsenic present in the water for consumption.

Figure 1. The arsenic biosensor exposed to arsenic grew pink while the arsenic biosensor not exposed to arsenic grew the normal white/yellow of E.coli. The clocks signify the rapid multiplication rate of bacteria.



Methods:

Refer to Figures 2 and 3 for detailed methods. The genes used for the arsenic biosensors are promoter J33201 to bind the arsenic and "turn on" the reporter E1010 which causes the red/pink color of the bacteria. Both genes are enclosed in the destination pSB1A3 which accounts for the Ampicillin resistance allowing for confirmation of the desired bacteria.

Results: We successfully constructed the arsenic biosensor that detects arsenate ($AsO4^{-}$) in the media. See figure 1 for the difference in growth between the sensor exposed to arsenic and the one not exposed. The sensor is currently very sensitive and can detect even 1ppb concentrations of arsenic in the media. The hope for future directions is to increase specificity of the promoter binding to the arsenic so that we can get a better picture of whether or no the water is safe for drinking.

Reaction Scheme

Figure 2. The genes used for the arsenic biosensors are promoter J33201 to bind the arsenic and "turn on" the reporter E1010 which causes the red/pink color of the bacteria.

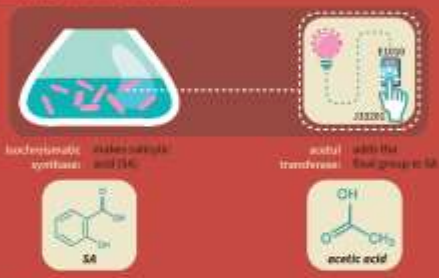
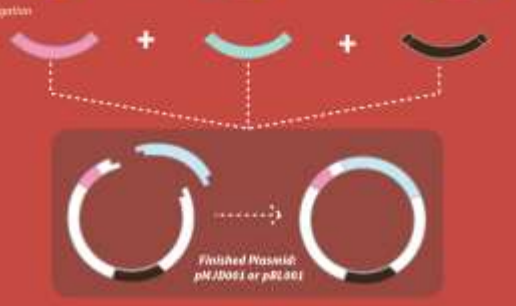


Figure 3.
Step 1: Enzym Digest

Step 2: Ligation



SOCIETAL IMPACT STATEMENT:
Biobricks can be utilized to build an infinite number of biological tools. Production of arsenic biosensors for identifying arsenic contamination will determine drinking water safety. Construction of aspirin producing bacteria is a potential alternative method to costly manufacturing procedures.

Aspirin Synthesizer

Intro:

Many medications are difficult and expensive to produce. The BioBricks Aspirin Synthesizer will use salicylic acid (SA), a normal bacterial waste product, and turn it into aspirin as the final waste product secreted by the E. coli. The idea behind the aspirin synthesizer is that if a simple small molecule drug can be made via E. coli then it is proof of concept of a possible route of drug manufacturing. Using biological systems would cut down on the complexity and cost of synthesizing a wide variety of medications. At this point the plan is to expand on that of the aspirin molecule to other medications that derive their structure from SA.

Figure 4. An isochorismate synthase is used to make the starting material SA and an acetyltransferase to put the finishing touch of an acetyl group to the SA to make and secrete the aspirin.

Methods:

Figure 1 and 3 show the detailed method of making the plasmid. The production of the plasmid is based off of the same protocol as the arsenic biosensor with the only difference being the plasmid gene parts used. Here an isochorismate synthase is used to make the starting material SA and an acetyltransferase will be used to put the finishing touch of an acetyl group on SA to make and secrete the aspirin.

Table 1. E.coli Growth Under Various Acetic Conditions

Conditions	No SA/ No AA	SA/ No AA	No SA/ AA	SA/ AA	Conc: SA
Growth (OD)	Yes	Yes	Yes	No.	No

Results: A concern of producing aspirin in bacteria is making the environment too acidic for the E. coli to live in. To address this problem, we ran several controls of the E. coli in acidic environments from salicylic acid and acetic acid. As shown in Table 1 the bacteria grew as normal in these environments confirming that we can proceed with further experiments to engineer the plasmid that will cause the E. coli to secrete aspirin. The goal of producing the Aspirin Synthesizer (pNJ0001) is for proof of concept that we can manufacture drugs via bacteria. We are planning to take this technology and add more genes to add different molecular groups to make different drugs. Irritable bowel syndrome (IBS) drugs are the next logical class of drugs to pursue because these drugs are direct salicylate derivatives.

References:

Roman Shrestha. Arsenic Contamination of Groundwater in Nepal: Good Public Health Intention
Gene Bad. Inquiries Journal.
Harish Arora, Sakib Pathan, and Saqib Kanori. Arsenic Pilot Plant Operation and Results-Weatherford, Oklahoma. Sandia Report. Printed May 2007.

Acknowledgements:

Thank you to the SWOSU Department of Chemistry and Physics, SWOSU College of Arts and Sciences, SWOSU Business Enterprise Center, and the US Department of Labor
for providing funding and support.
Thank you to Micah Morehart for assistance with experiments.

2018 Research Day at the Capitol

INSIGHT FROM A JUDGE

Sherry Marshall

President & CEO, Science Museum Oklahoma

Educational background: Physics, with additional emphasis in Chemistry, Applied Behavioral Science in Education, and Curriculum and Instruction