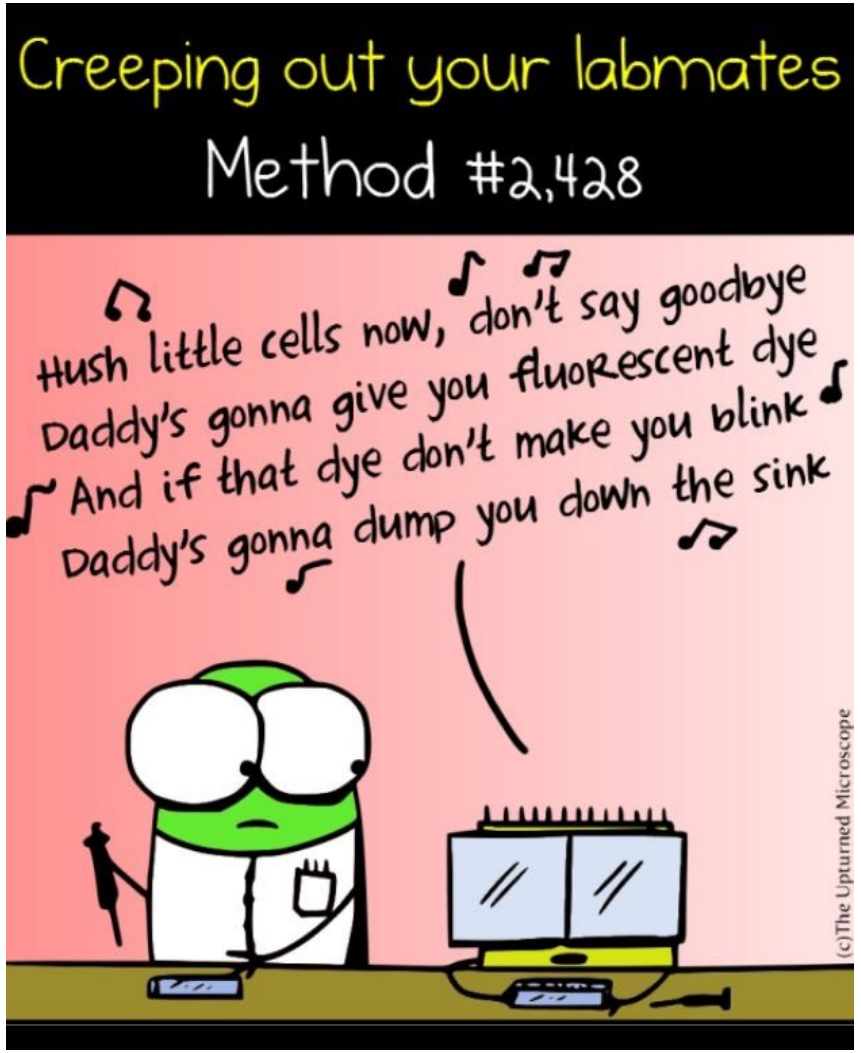
M1D2: Prepare and treat cells for foci experiment

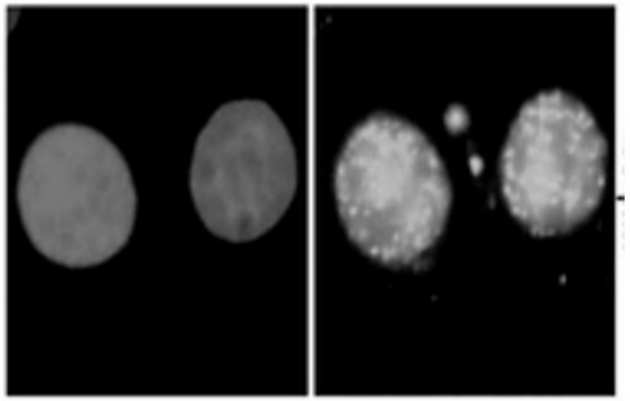
1. Prelab
2. Experiments for today:
   - Adhere cells to coverslips
   - Treat and fix MCL-5 cells for H2AX assay



Admin Notes:

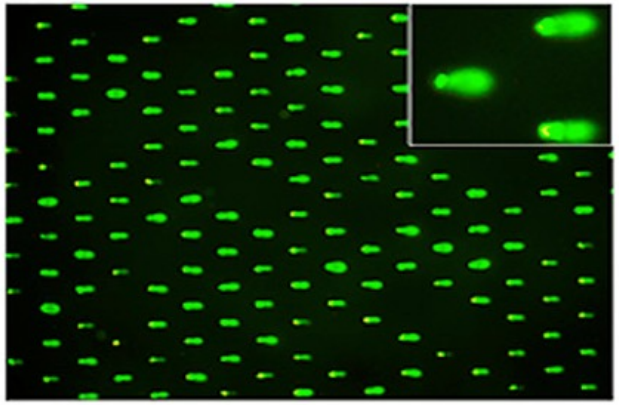
If you haven't already, respond to Noreen's office hours email

Mod1 Overview



1. Use repair foci experiment to measure DNA breaks

- Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γH2AX foci formation



2. Use high-throughput genome damage assay to measure DNA damage

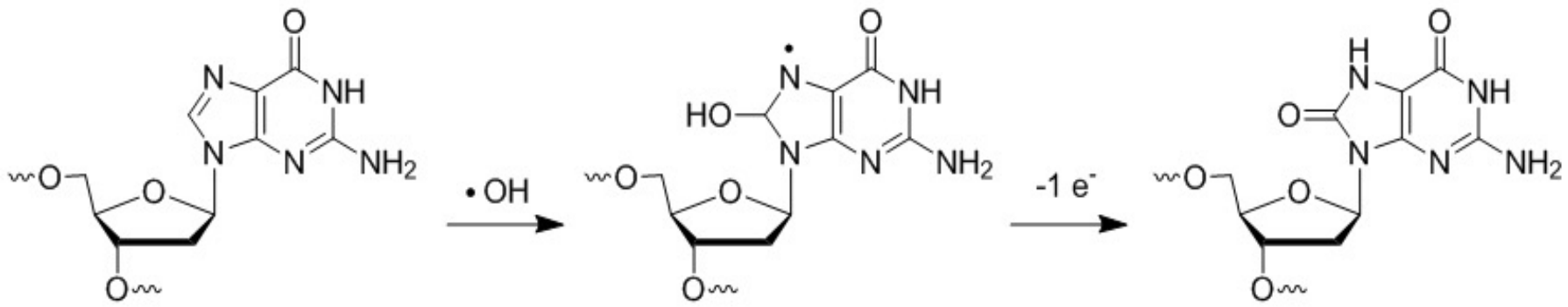
- Measure effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

How does H_2O_2 damage DNA?

ROS = _____

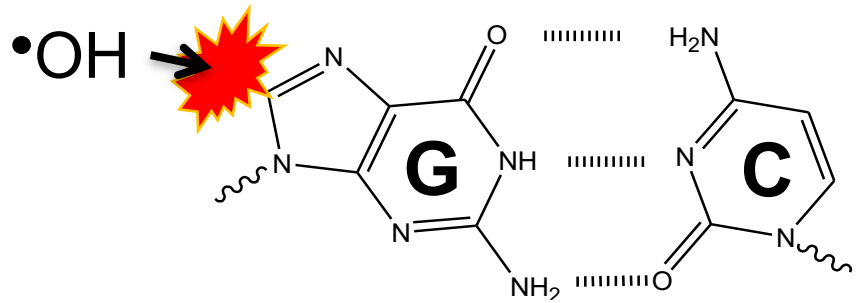
$H_2O_2 + e^- \rightarrow HO^- + \bullet OH$

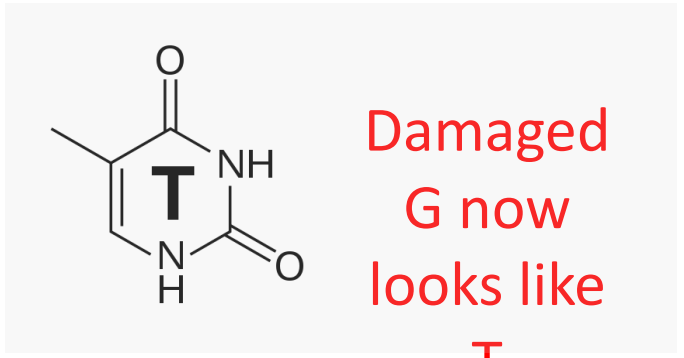
$2\ H^+ + 2\ e^- + H_2O_2 \rightarrow 2\ H_2O$

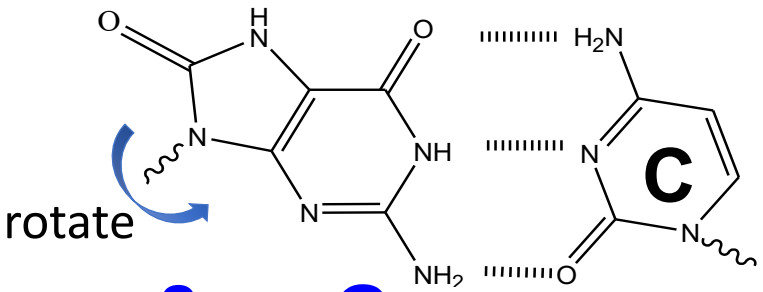


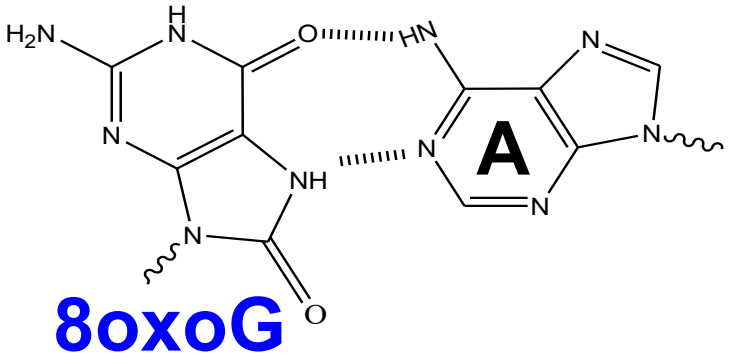
Guanine **8-oxo-Guanine**

How does H_2O_2 damage DNA?





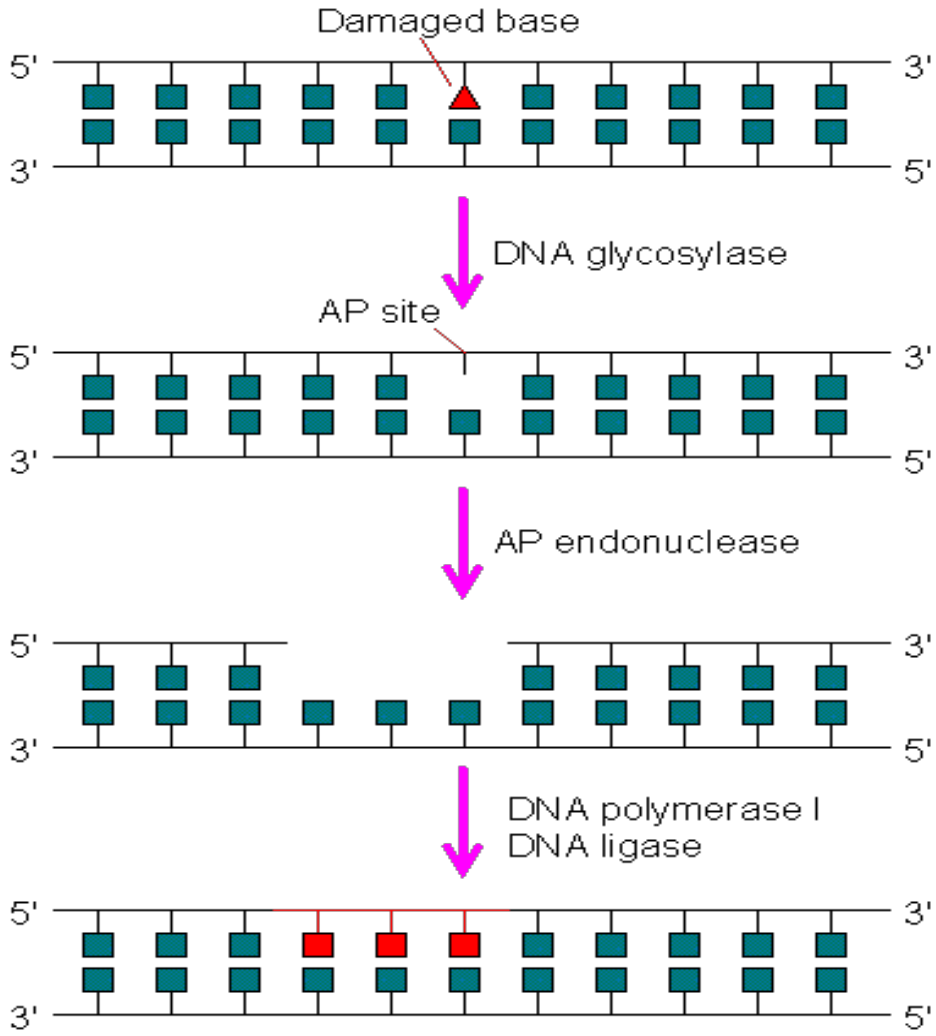




Mutation if replicated

How do cells repair oxidative DNA Damage?

Typically, the BER pathway



http://www.web-books.com/MoBio/Free/Ch7G.htm

How do we look at DNA damage in intact cells?

Look for γ -H2AX foci

H_2O_2 can cause damage to DNA, resulting in a damaged base

↓

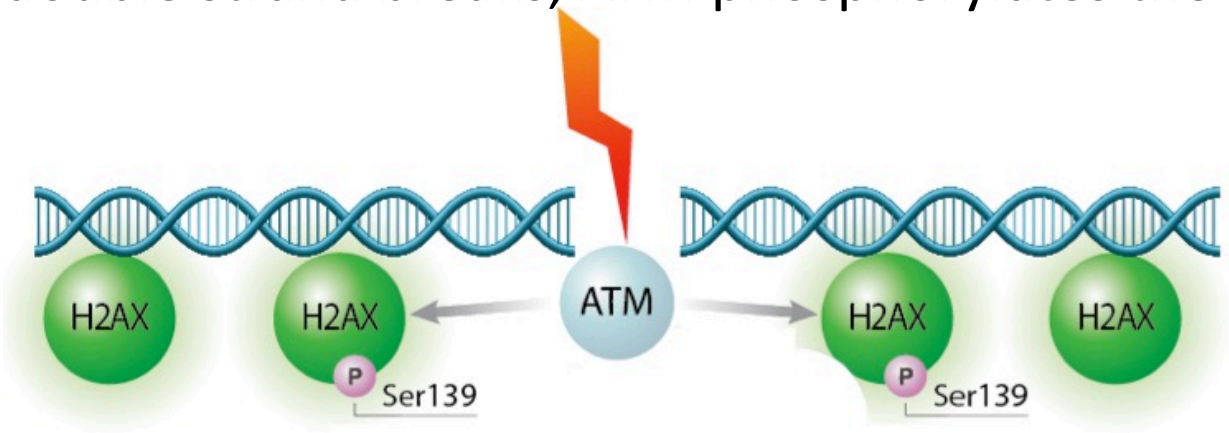
That damage causes a single strand break as cell tries to repair the DNA

↓

Multiple single strand breaks cause double strand breaks

↓

At double strand breaks, ATM phosphorylates the histone H2AX



We can identify the frequency of these DSBs as a measure of DNA damage

Treatment conditions for this experiment

- Goal: identify any additive effect pretreatment with As has on H_2O_2 induced DNA damage
  - Treat cells with As for 24 hours, then treat cells with H_2O_2 for 30 minutes

Experimental Condition

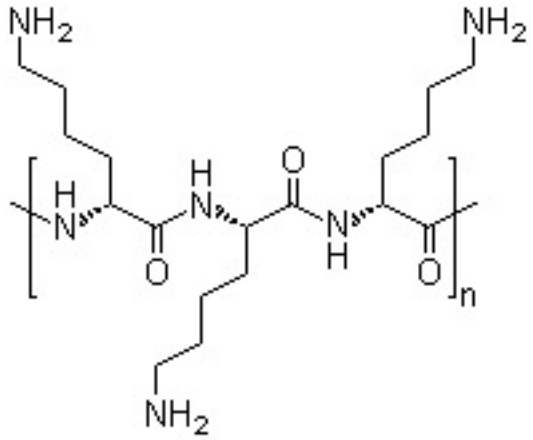
Control Conditions

Our imaging protocol requires cells to adhere to glass coverslips in monolayer

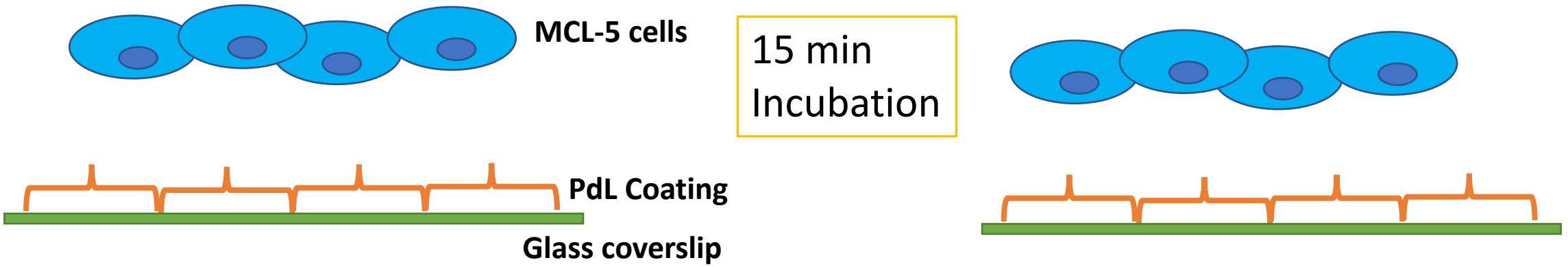
- We have non-adherent cells!
- Must adhere them to coverslips prior to imaging

Poly-d-Lysine

- Cannot image **γ** -H2AX foci with cells in suspension
  - Want clear images of nuclei
  - Immobilize cells in a monolayer on glass coverslips



- Many ways to get cells to adhere to glass or plastic
  - ECM molecules (like laminin)
  - Charged polymer molecules (like PdL)
  - Biological substrates (like Polyphenolic Proteins secreted by marine mussels)



In lab today

1. Expose As-treated cells to H_2O_2 and fix them for IF staining
2. Talk about purpose and structure of background and motivation

DO NOT use the aspirator to remove the cell media.
It needs to be collected as hazardous waste!

M1D3HW

1. Work **with your lab partner** to write methods for M1D1 and M1D2
2. Schedule appointment with BE Comm Fellow before M1D5

Pro tips for writing a methods section

Include enough information to replicate the experiment

- List manufacturer's name (Company)
- Be **concise and clear** in your description

Use subsections with descriptive titles

- Put in logical order, rather than chronological order
- Begin with topic sentence to introduce purpose / goal of each experimental procedure

Use clear and concise full sentences

- NO tables or lists, all information should be provided in full sentences and paragraphs
- Write in passive voice and use past tense

Use the most flexible units

- Write concentrations (when known) rather than volumes

Eliminate 20.109 specific details

- Example "labeled Row A, Row B…"
- Do not include details about tubes and water!
- Assume reader has some biology experience
- Include parts of the protocol that the teaching faculty completed, but do not say "completed by teaching faculty."

How can you improve this example?

"Cells were grown in 12 mL of RMPI supplemented with FBS. We spun down the cells and counted them with a hemocytometer. Flasks were incubated in 37 C incubator."

How can you improve this example?

What cells? From where were the cells attained?

How much? What else was added to the media?

"Cells were grown in 12 mL of RPMI supplemented with FBS. We spun

Volume here does not have context as based on the flask used. When might flask / plate size be helpful??

Define all abbreviations and include supplier / manufacturer.

Use passive voice and avoid jargon!

down the cells and counted them with a hemocytometer. Flasks

Be specific about the purpose of each of the steps used…cells were harvested using centrifugation (be sure to include speed and time) then counted using a hemocytometer. And what else was used? At what final concentration / percent?

Be specific about the subject of each action / step.

were incubated in 37 C incubator."

Specific location / equipment used is not important, just the temperature conditions. What other growth conditions were maintained?

Revised example…

Maintaining MCL-5 cell line

Human lymphoblastoid cells (MCL-5) cells (gift of Engelward Laboratory, MIT) were grown in Roswell… (RPMI) (Manufacturer) supplemented with 10% fetal bovine serum (FBS) (Manufacturer) and 100 U / mL of penicillin and streptomycin (Manufacturer). To harvest, cells were centrifuged for 5 minutes at 300g and pelleted cells were resuspended in fresh media. Cells were counted using 10% (v/v) trypan blue and a hemocytometer. Cultures were maintained at 37 C, 5% CO_2, and 95% relative humidity.

What is the purpose of the Background & Motivation section?

What *specific* information should be included in the Background & Motivation section?

Notes on Background & Motivation section…

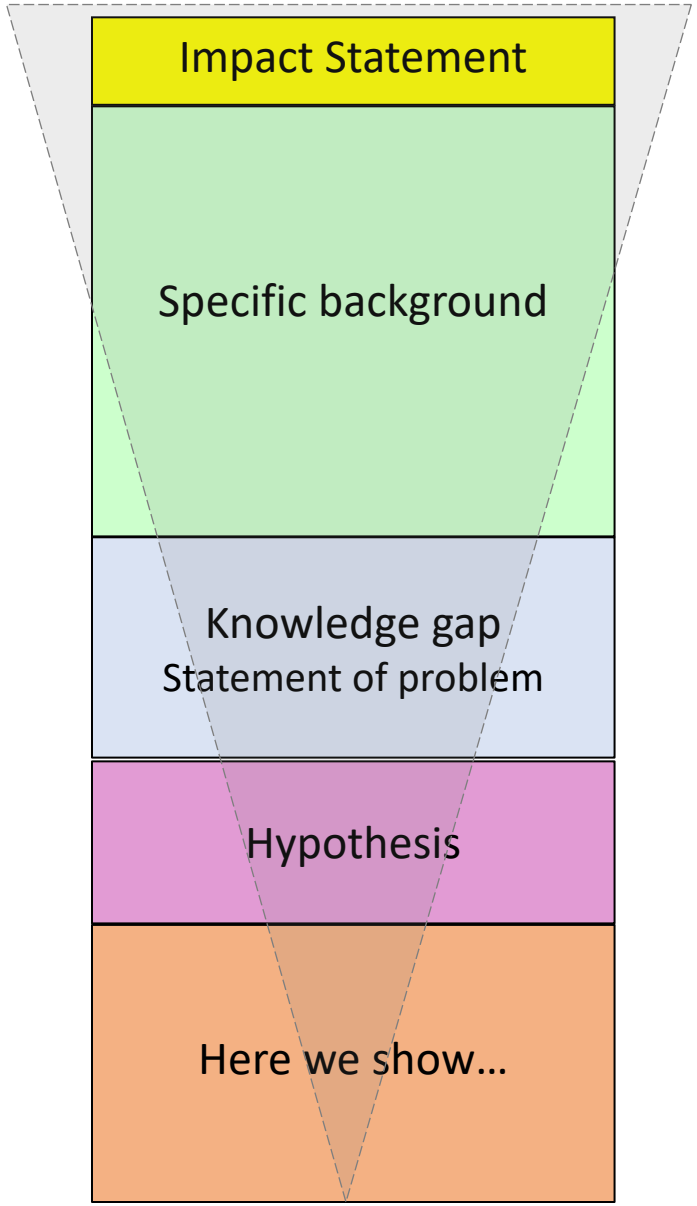
- Anchor your research in a general topic that is important to a broad audience
  - Focus on describing what is currently known in the field
  - Reference the relevant research in the field
- Connect your research to the general topic
  - Minimum essential information
  - Introduce specific technologies necessary for understanding your specific project
- Address how you will expand on what is currently known
  - Include evidence of incompleteness of current understanding
  - Motivate your investigation
  - Include a clear hypothesis / research goal
- Provide a preview of your findings and the implications
  - Tie back to the initial general topic
  - Avoid including extensive methods details

Notes on topic sentences…

- Topic sentence = first sentence of each paragraph

- Should ‘funnel’ from big picture topic to your specific research question / project
  - Provide only the background needed to understand research / problem / goal
  - Clearly state what is not currently known
  - Address how you will fill knowledge gap
  - Provide preview of your results

- Include references!!



How should you introduce your story?

1st paragraph: what is the big picture / problem?

2nd paragraph: what is currently known?

3rd (or 4th) paragraph: what is your research question?

4th (or 3rd) paragraph: how will you address your question?

5th paragraph: here we show…