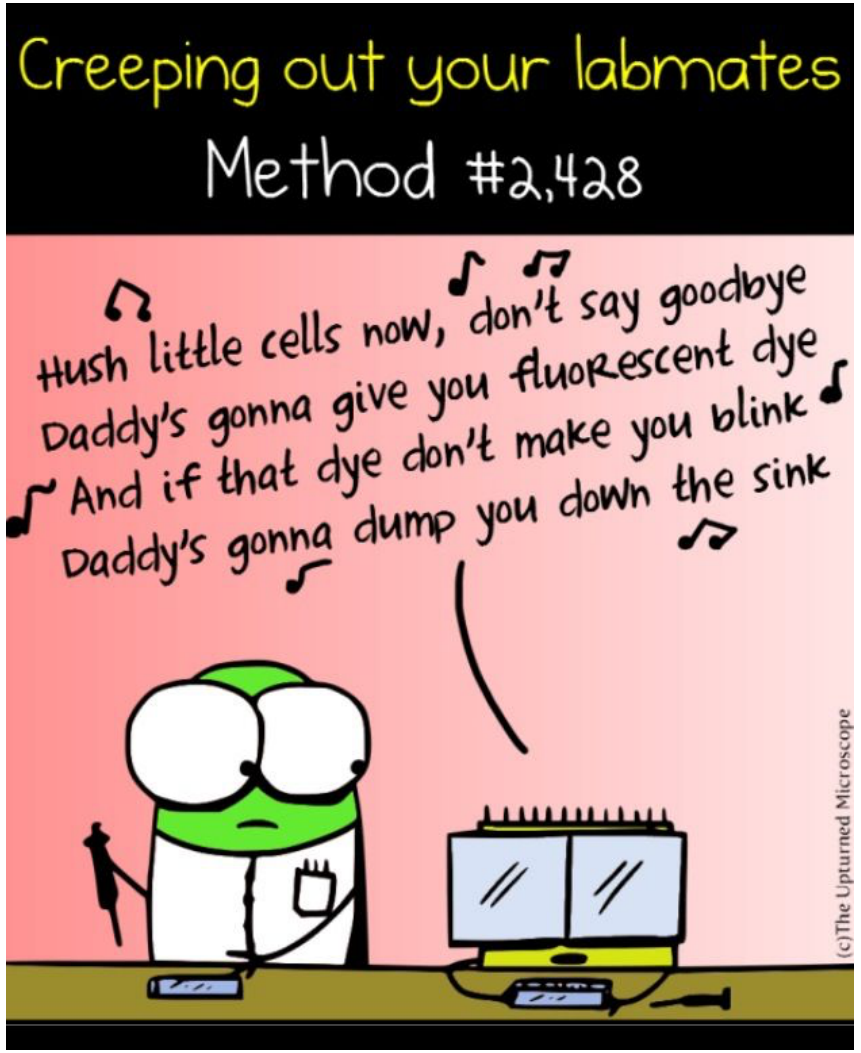
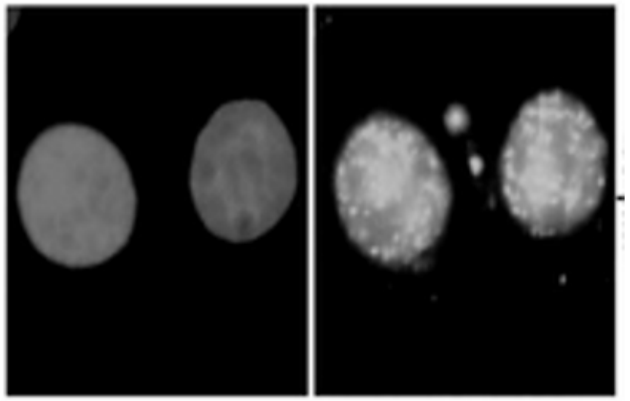
M1D2: Prepare and treat cells for foci experiment

1. Prelab
2. Experiments for today:
   - Demo of coverslip coating
   - Treat and fix MCL-5 cells for H2AX assay

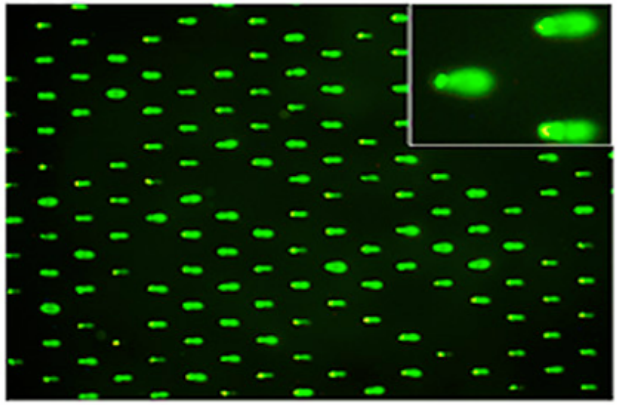


Mod1 Overview



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

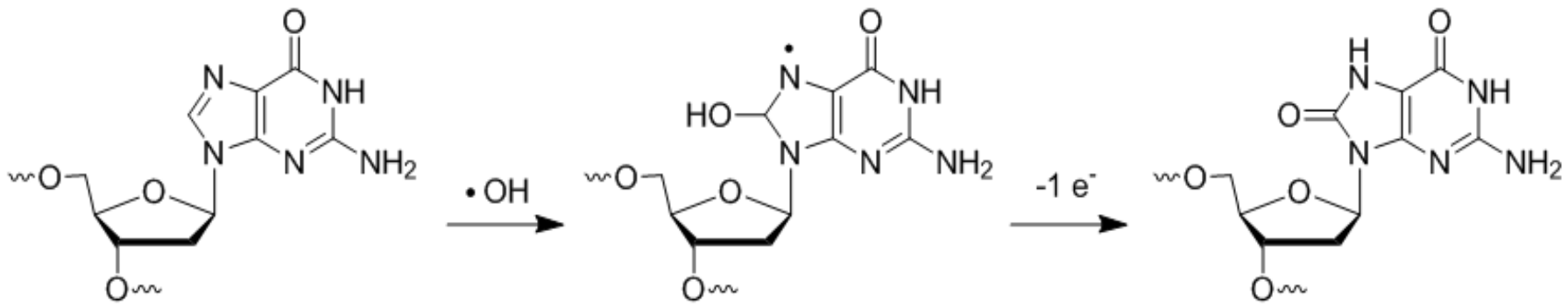
- Measure effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

How does H_2O_2 damage DNA?

ROS = _____

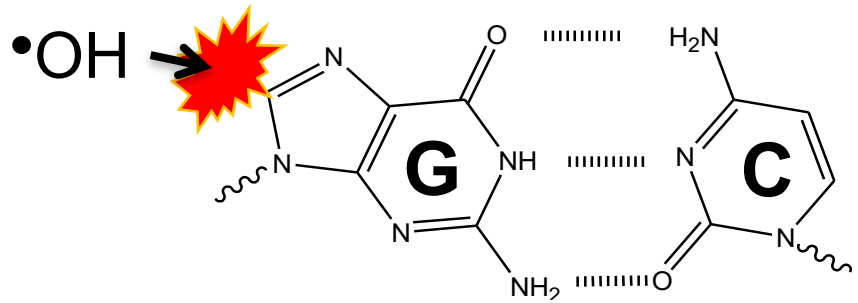
$H_2O_2 + e^- \rightarrow HO^- + \bullet OH$

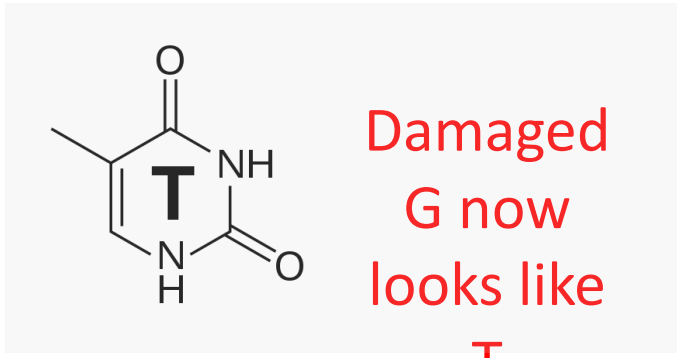
$2\ H^+ + 2\ e^- + H_2O_2 \rightarrow 2\ H_2O$

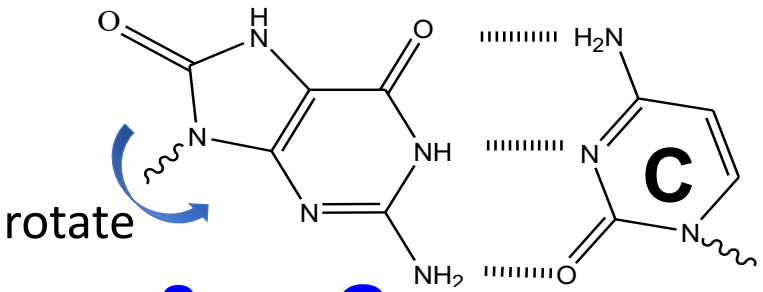


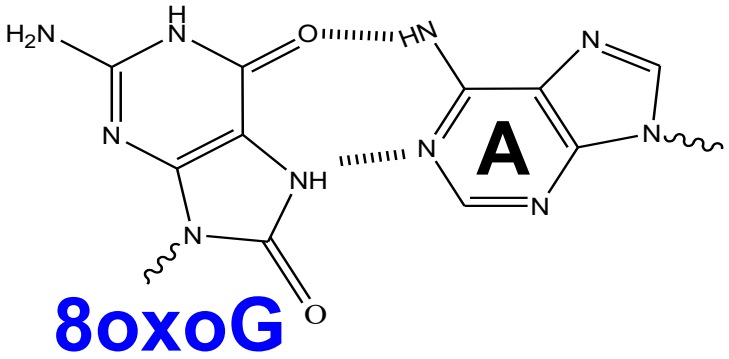
Guanine **8-oxo-Guanine**

How does H_2O_2 damage DNA?





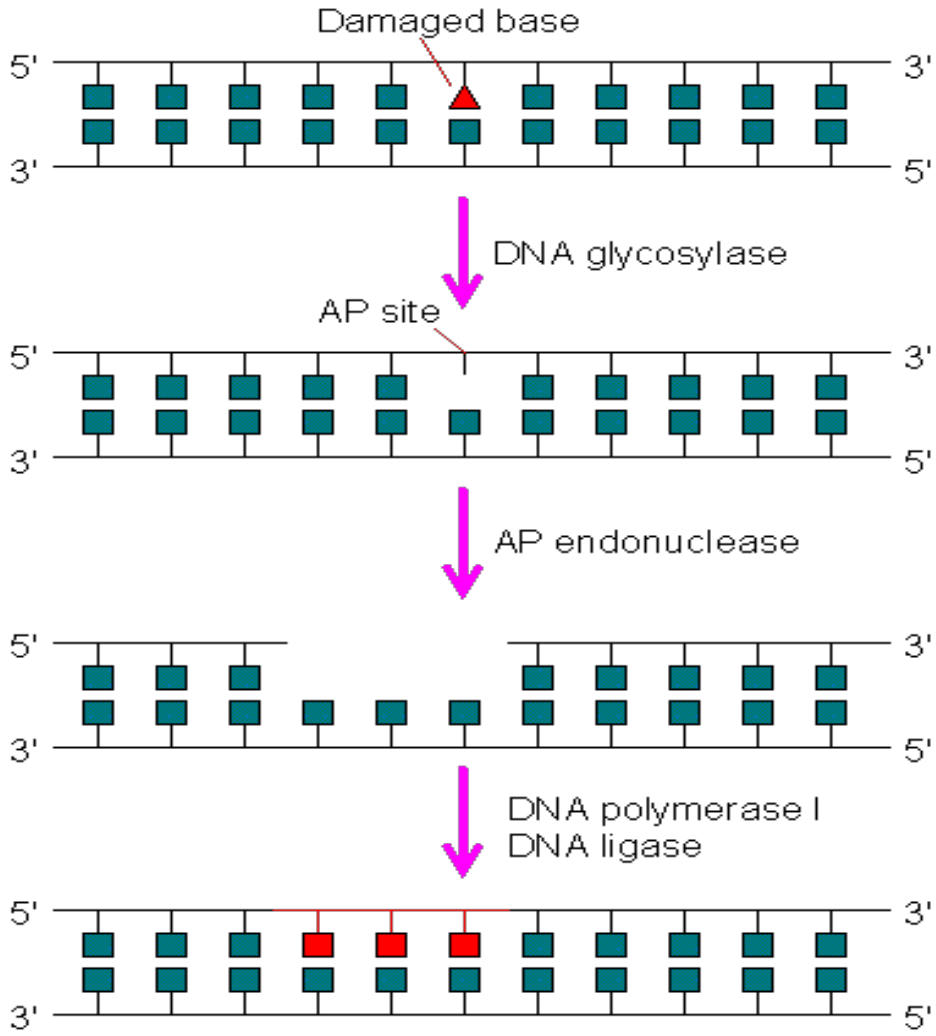




Mutation if replicated

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

↓

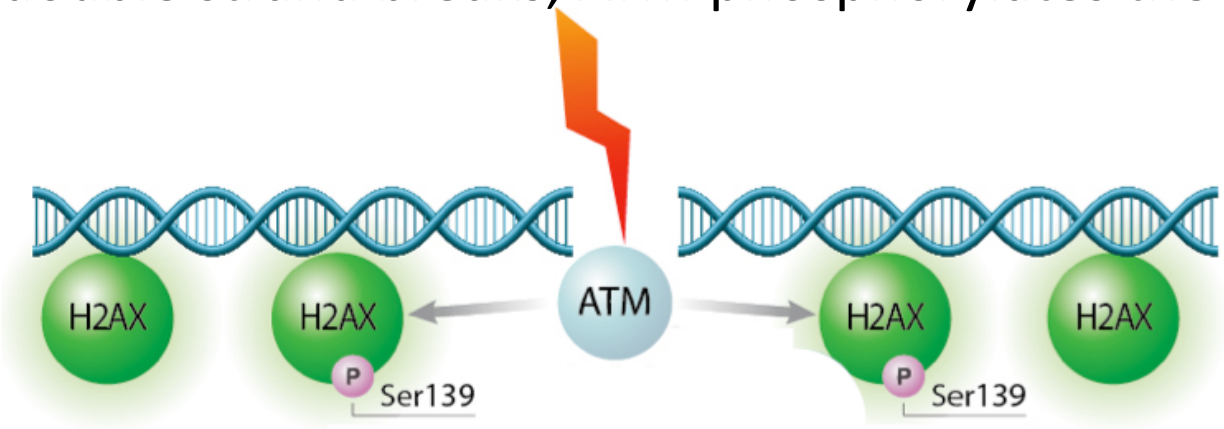
That damage causes a single strand break as cell tries to repair the DNA

↓

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

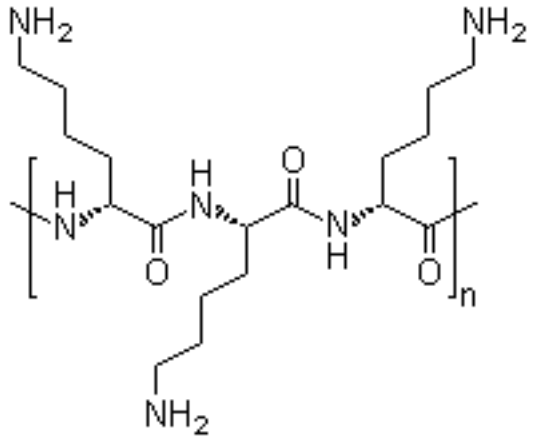
Control Conditions

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

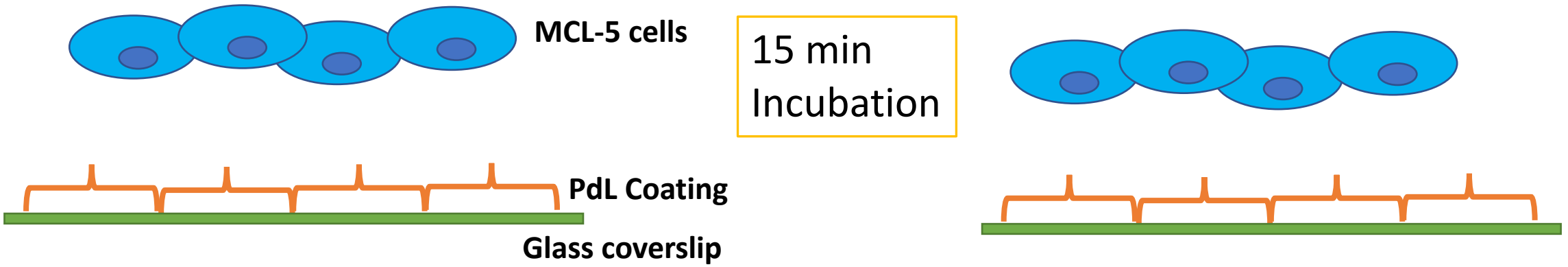
- We have non-adherent cells!
- Must adhere them to coverslips prior to imaging

Poly-d-Lysine

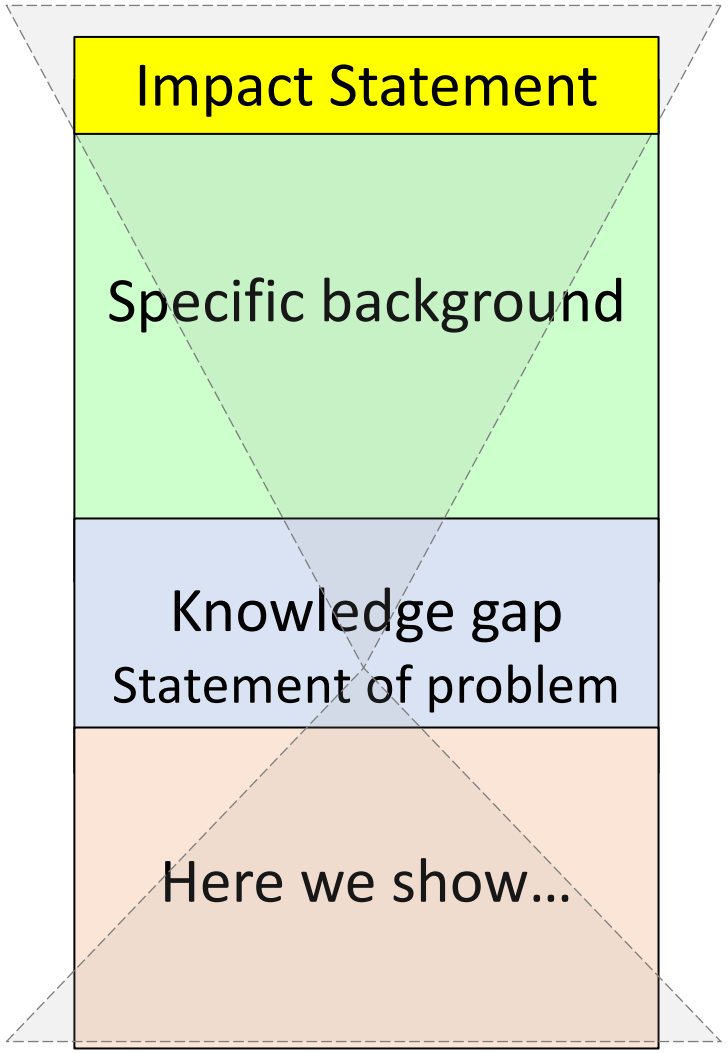
- Cannot image **γ** -H2AX foci with cells in suspension
  - Want clear images of nuclei
  - Immobilize cells in a monolayer on glass coverslips



- 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)

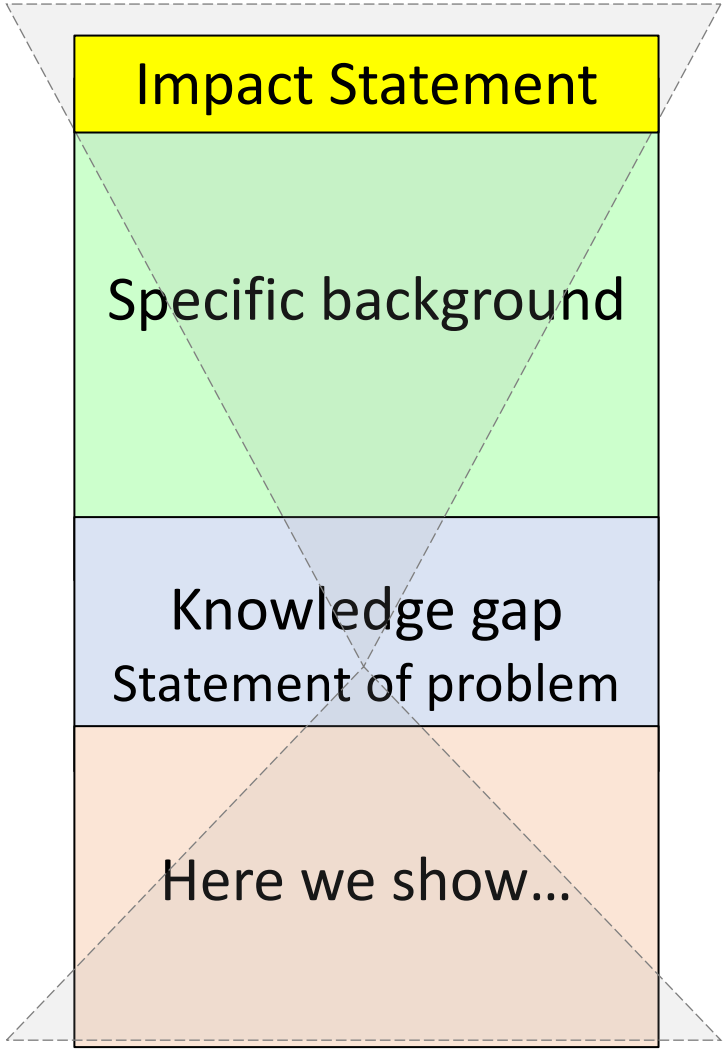


What goes into a background/motivation section?

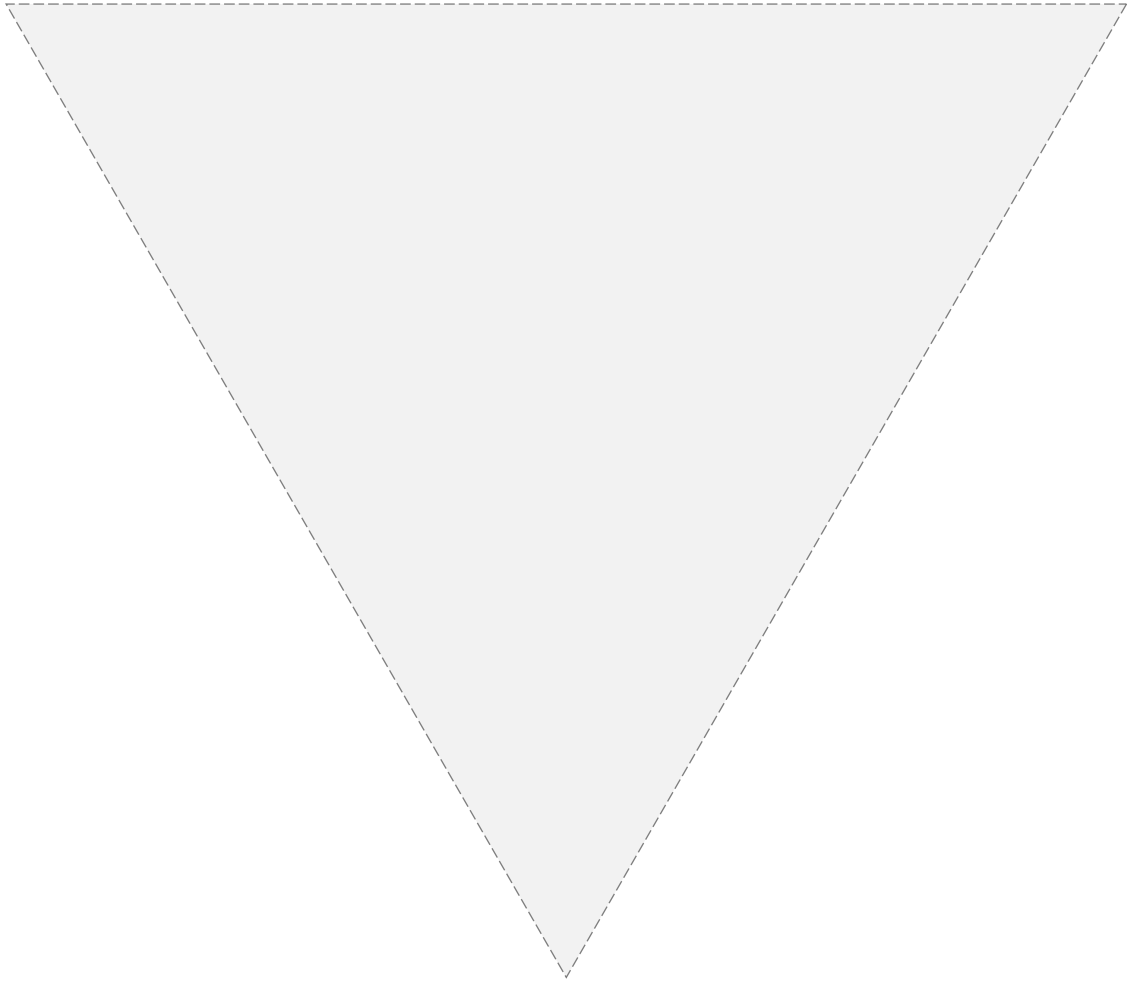


- Your research is anchored in a general topic that your audience cares about or could be interested in.
  - Focus on describing previous work in the field
- Specific background connects your project with the general background.
  - Minimum essential information
  - References current work in the field
  - Introduce specific technologies necessary for understanding the project
- The question you address is clearly articulated, connected to the background, and has appropriate scope for the project
  - Give evidence of incompleteness of current understanding, therefore motivating the investigation
  - Include a space holder for your hypothesis (or come up with one)
- A preview of your findings and their implications
  - Light on Methods

What goes into your introduction?



Choose one narrative



In lab today

1. Work through wiki to treat and fix MCL-5 cells in preparation for **γ** -H2AX immunofluorescent staining

M1D3HW

1. Write topic sentences (1st sentence) to outline your Data Summary's Background and Motivation section (3-5 sentences total)
   - Remember to include references with summary & why you chose it
2. Read paper on wiki to prepare for in-class discussion
3. Schedule appointment with BE Comm Fellow before M1D5