MIT BE
BIOLOGICAL ENGINEERING

Communication Lab

# 20.109 Communication Workshop 4

# Research Manuscripts: structure and writing process

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# Reflecting on Journal Club

What did other people do well that helped you understand their presentations?

What might you do differently in future presentations?

# There are no explicit models for successful papers.

When you read a paper or sentence you like, collect it!

favorite papers

Analyze what makes it work.

Try using their techniques.

# Writing a paper integrates topics we have already covered…

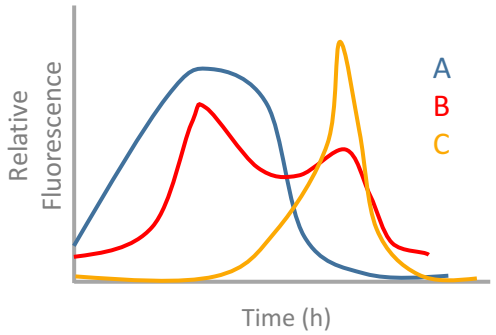
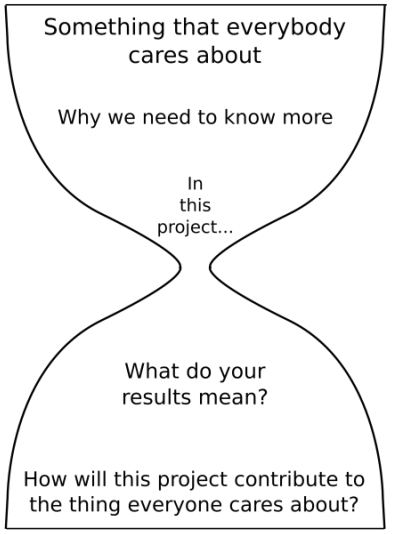


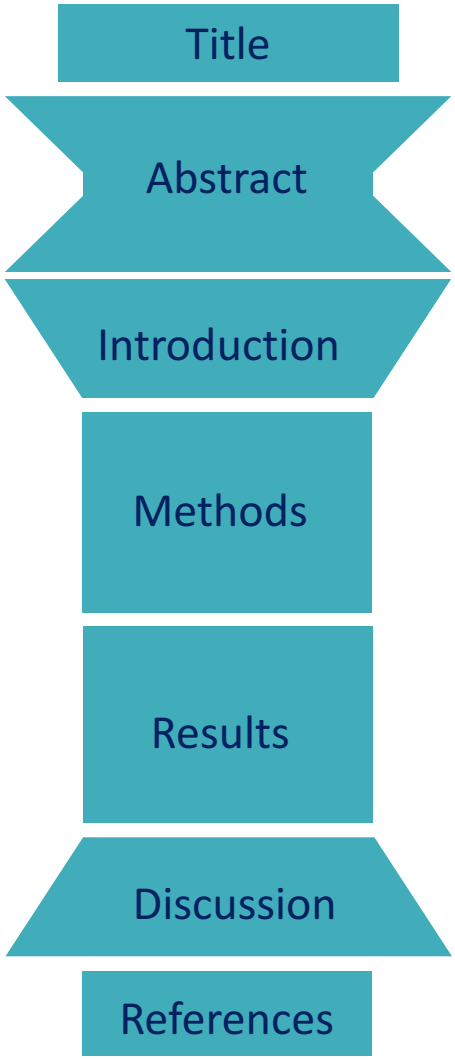

**Fig. 1: A, B, and C have different dynamics under Condition X.** A, B, and C were sampled using Method 1 and their fluorescence quantified with Method 2. Fluorescence data normalized to negative control.

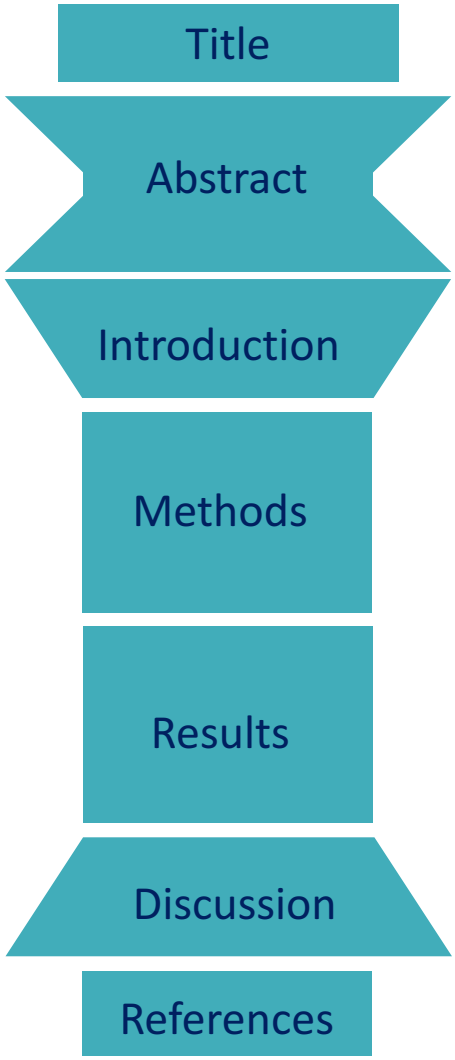



Workshop #1
Figures & Captions

Workshop #2
Abstracts & Titles

# A typical paper has sections to guide us

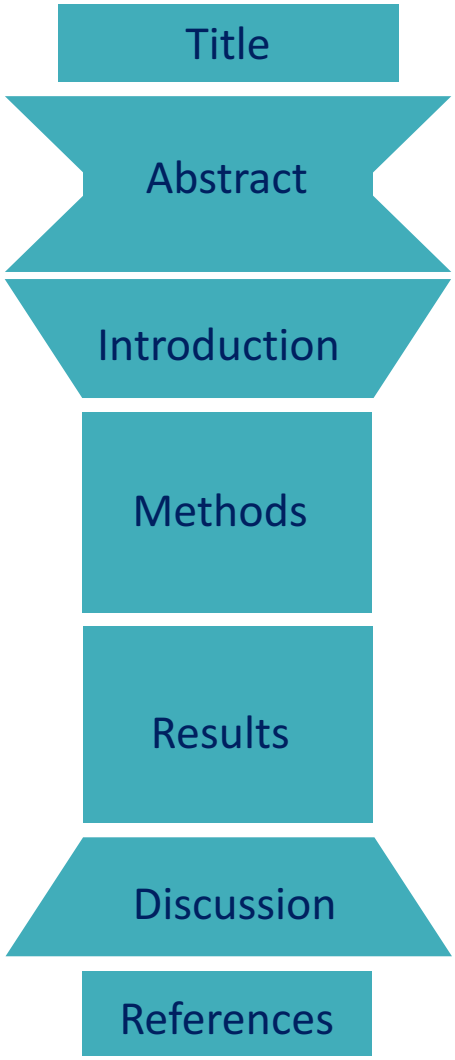





In what order do you *read* a paper?

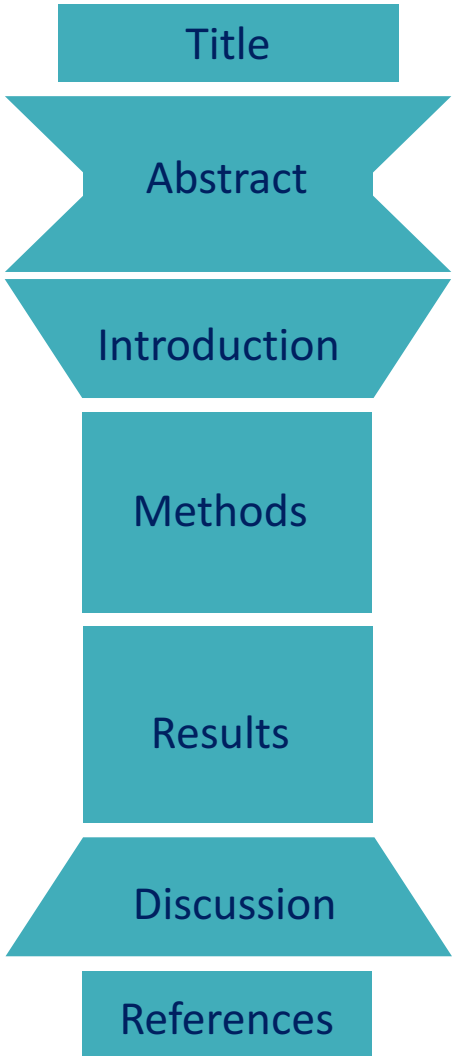
In what order will you *write* a paper?

# Papers are often thought of as linear…




…yet are both **read** and **written** nonlinearly.

# We recommend the following order for writing your paper:



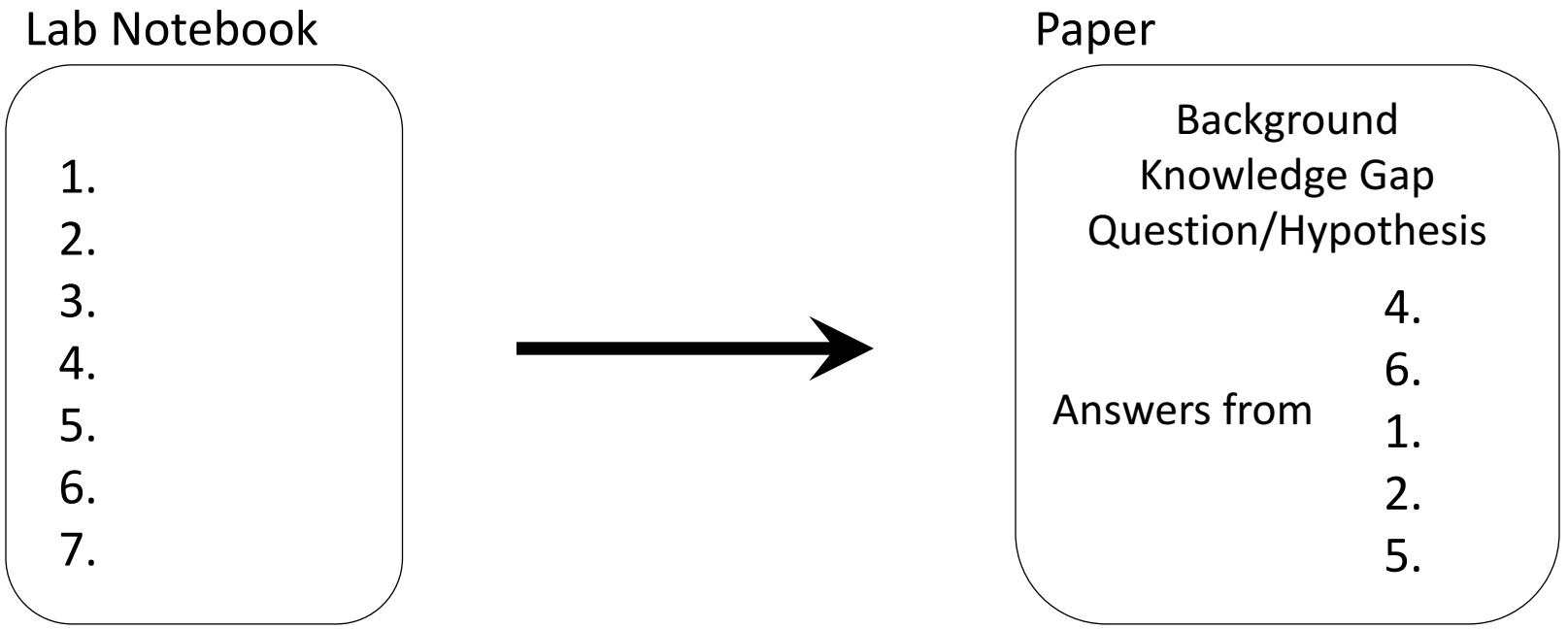

1. Figures + Captions
2. Methods
3. Results
4. Discussion
5. Introduction
6. Abstract
7. Title

# Revising papers is essential

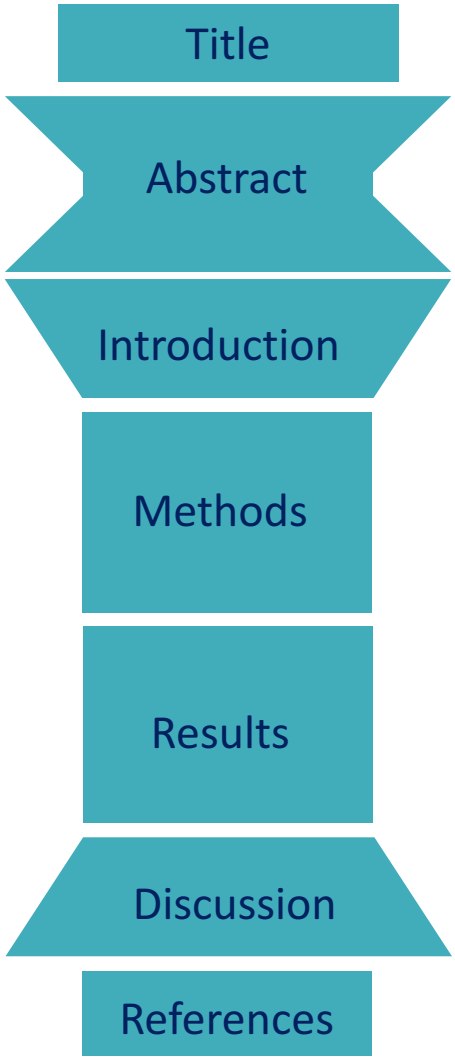
- Do not try to write this paper in one day.

- **Outline** or draft the sections, then set aside the paper for several days.
- When you get stuck: write topic sentences, work on the next section, look at examples
- Get feedback: peers, instructors, Comm Lab!

# Your paper tells a story about your data



# The sections of your paper answer different questions




What is your story?
Why should I care?

What do I need to know to understand your story?

How did you do your experiments?

What did you find?

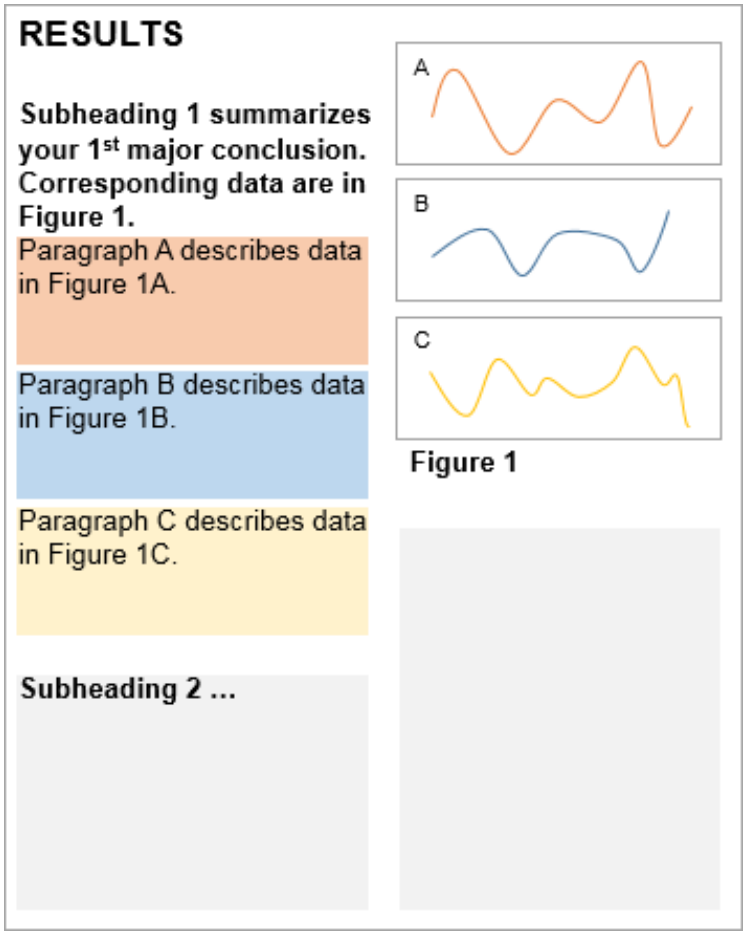
What does it mean?
Where can we go from here?

# Redundancy in your paper helps your reader find the information they need.

| | | |
|---|---|---|
| General background | Something everyone in your audience cares about. | Introduction: beginning |
| Specific background | Zoom in from General Background to the thing you did. | Introduction: middle |
| Knowledge gap, Unknown | Question that will be answered by your research. Problem, phenomenon that is not understood. | Introduction: end |
| HERE WE SHOW | Conclusion, answer to the Unknown | Introduction: end<br>Results: end<br>Discussion: beginning |
| Results | Brief summary of approach + very high-level results.<br>Common pitfall = too much Methods/Results. | Introduction (high level)<br>Results (high level)<br>Methods |
| Implication, Significance | So what?<br>What do your results mean for the thing everyone cares about? | Discussion |

# Use parallelism: Put all of your content in the same order.

Data ◆ Methods ◆ Results ◆ Discussion

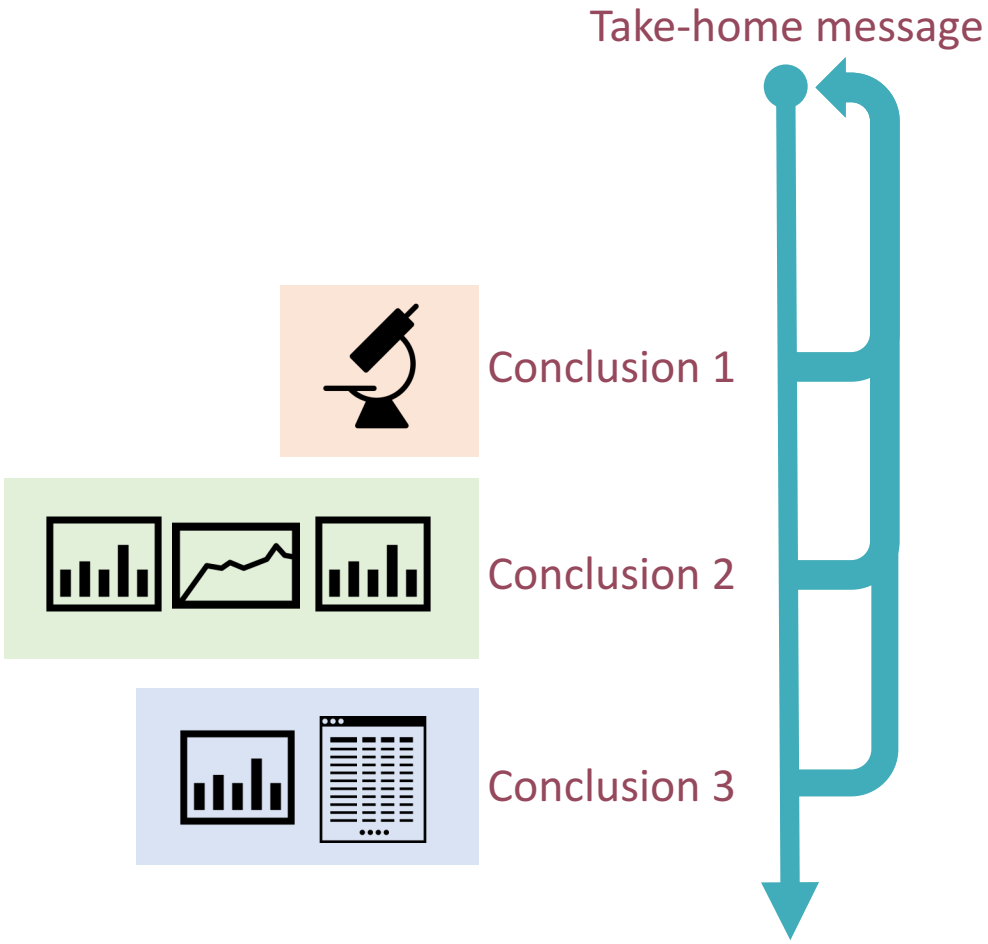



Methods: Most experimental detail

Results: Motivation for key methods you used; high-level summary of methods used to obtain results

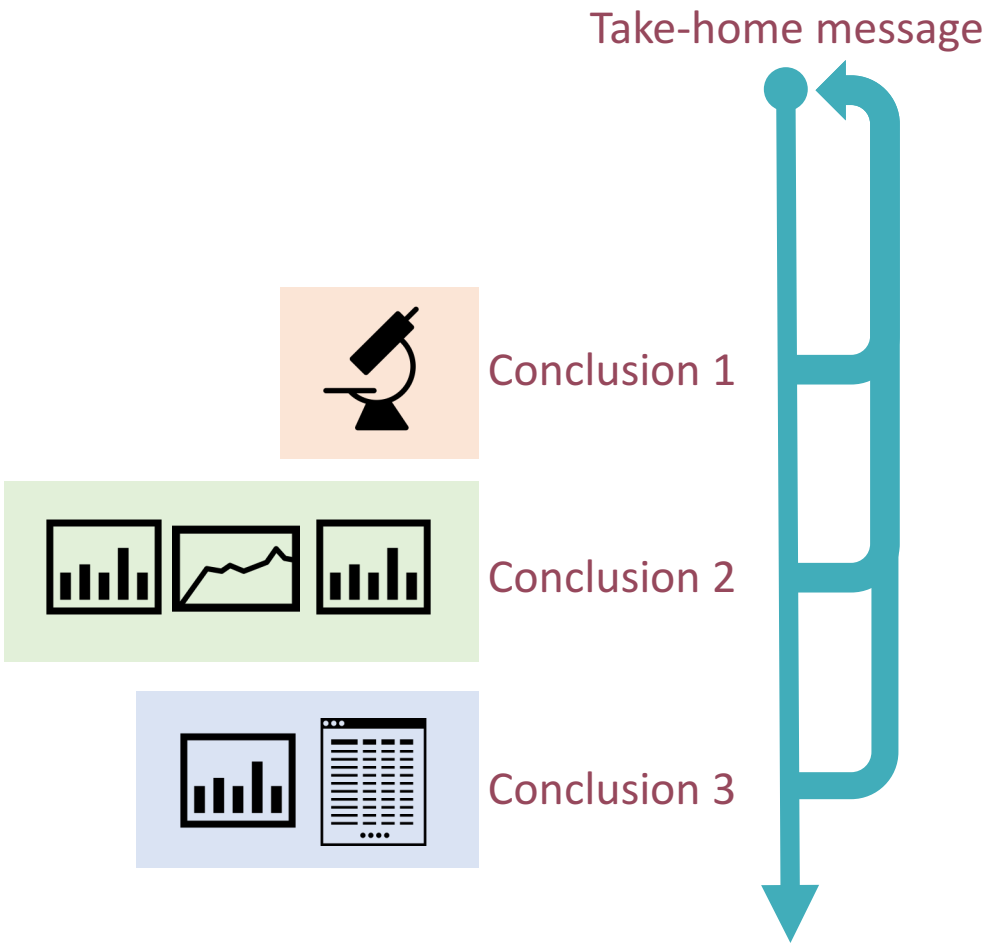
Figure captions: high-level description of methods used

# Figures: Identify a take-home message and key conclusions through your figures



# Paper structure: Results

# Results = rationale + data + conclusion



# Results = rationale + data + conclusion

Conclusion 1

Conclusion 2

Conclusion 3

**RESULTS**

**Subheading 1 summarizes your 1st major conclusion. Corresponding data are in Figure 1.**

Paragraph A describes data in Figure 1A.

Paragraph B describes data in Figure 1B.

Paragraph C describes data in Figure 1C.

**Subheading 2 ...**

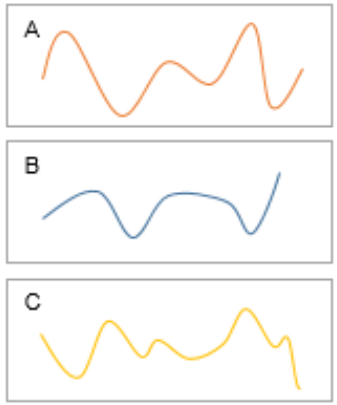



**Figure 1**

# Results = rationale + data + conclusion

Conclusion 2

Title

Paragraphs for each of the key experiments

**RESULTS**

**Subheading 1 summarizes your 1st major conclusion. Corresponding data are in Figure 1.**

Paragraph A describes data in Figure 1A.

Paragraph B describes data in Figure 1B.

Paragraph C describes data in Figure 1C.

**Subheading 2 ...**

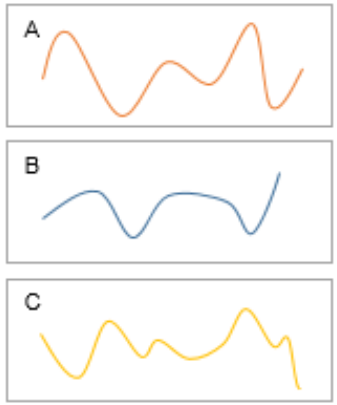
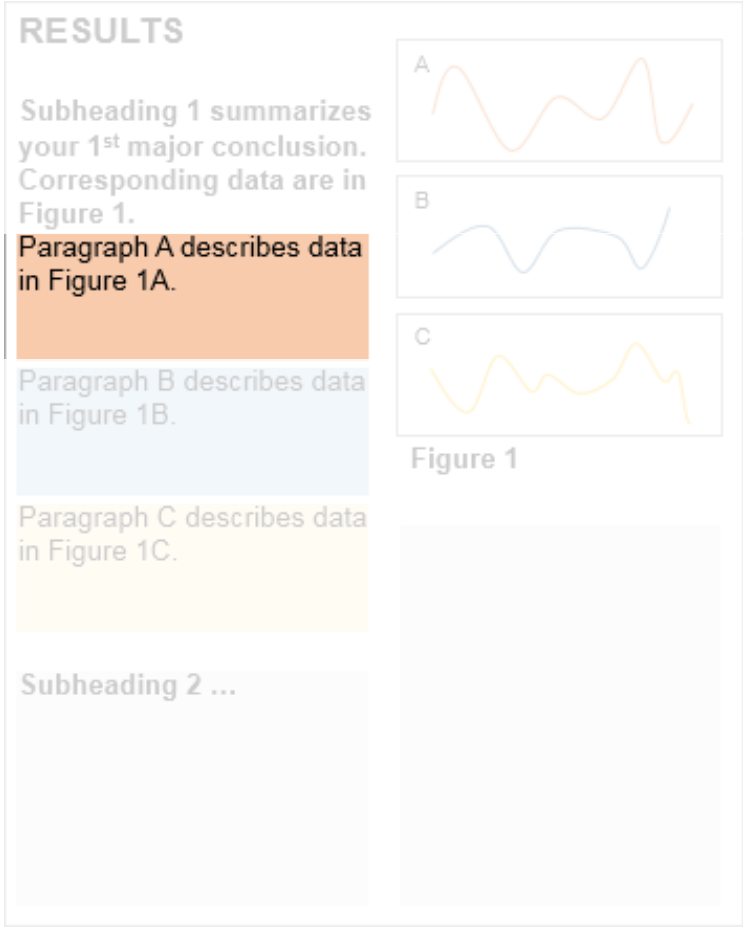



**Figure 1**

# Results = rationale + data + conclusion



# Results = rationale + data + conclusion

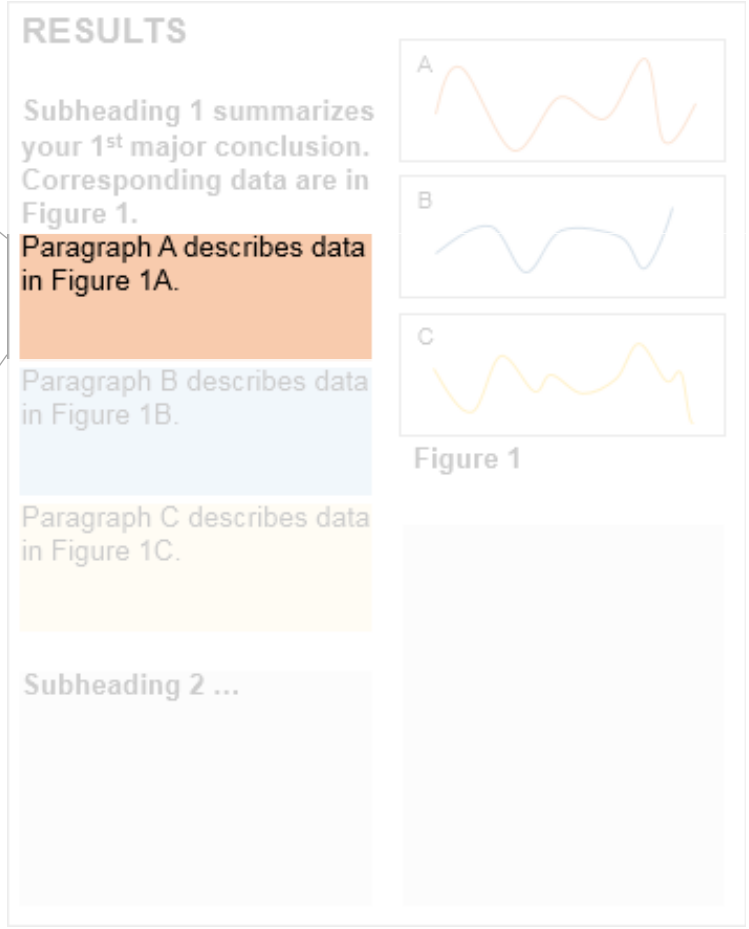
**In order to determine *X*, *Y* was performed, showing *Z* major results.**

**Data + conclusions**

- pro, then con
- most to least important
- experiment vs. control

**Transition sentence**

- re-summarize findings
- justify movement to next experiment or hypothesis



# Results: Cover minimal essential data.

Maximize signal-to-noise.

Include

- The experiment or dataset that is the **strongest proof** of your conclusion.
- Parts of your chosen dataset might contradict your main conclusion, or support one claim but not another.
- Discuss all parts of a figure in your results section.

# Results: Cover minimal essential data.

Maximize signal-to-noise.

Usually put in Supplement*

*109 paper has no supplementary section

Experiments or datasets that…

- Also support your conclusion but are not the strongest proof

  method is less validated
  data are less statistically significant
  data are less intuitive to interpret

- Were run to validate methods

- Were run to rule out alternative hypotheses

# Results: Follow the Herskowitz Rule

**time** spent describing a result $\propto$ **importance** of that result to the paper's main conclusion

Prof. Ira Herskowitz
(1946-2003)

# Results: Let's look at an example

Each section corresponds to one figure

Your

Zetsche et al, 2015. *Cell*, 163.

## Results:

Let's look at right side of p. 762 & Figure 4

### The RuvC-like Domain of Cpf1 Mediates RNA-Guided DNA Cleavage

### Figure 4. Catalytic Residues in the C-Terminal RuvC Domain of FnCpf1 Are Required for DNA Cleavage

*What do you notice about these titles?*

Zetsche et al, 2015. *Cell*, 163

## Results: Motivation of experiments
First sentence of this result:

The RuvC-like domain of Cpf1 retains all of the catalytic residues of this family of endonucleases (Figures 4A and S4) and is thus predicted to be an active nuclease.

*What do you think they will test in this experiment?*

Zetsche et al, 2015. *Cell*, 163.

# Results: Motivation leads to what was done (not so much how)

The RuvC-like domain of Cpf1 retains all of the catalytic residues of this family of endonucleases (Figures 4A and S4) and is thus predicted to be an active nuclease. Therefore, we generated three mutants—FnCpf1(D917A), FnCpf1(E1006A), and FnCpf1(D1225A) (Figure 4A)—to test whether the conserved catalytic residues are essential for the nuclease activity of FnCpf1.

Zetsche et al, 2015. *Cell*, 163.

# Results: Example of data, then conclusions

We found that the D917A and E1006A mutations completely inactivated the DNA cleavage activity of FnCpf1, and D1255A significantly reduced nucleolytic activity (Figure 4B).

Zetsche et al, 2015. *Cell*, 163.

# Results: Example of data, then conclusions

We found that the D917A and E1006A mutations completely inactivated the DNA cleavage activity of FnCpf1, and D1255A significantly reduced nucleolytic activity (Figure 4B). These results are in contrast to the mutagenesis results for Streptococcus pyogenes Cas9 (SpCas9), where mutation of the RuvC (D10A) and HNH (N863A) nuclease domains converts SpCas9 into a DNA nickase […].

Zetsche et al, 2015. *Cell*, 163.

# Results: Example of data and conclusions

We found that the D917A and E1006A mutations completely inactivated the DNA cleavage activity of FnCpf1, and D1255A significantly reduced nucleolytic activity (Figure 4B). These results are in contrast to the mutagenesis results for Streptococcus pyogenes Cas9 (SpCas9), where mutation of the RuvC (D10A) and HNH (N863A) nuclease domains converts SpCas9 into a DNA nickase […]. These findings suggest that the RuvC-like domain of FnCpf1 cleaves both strands of the target DNA, perhaps in a dimeric configuration. Interestingly, size-exclusion gel filtration of FnCpf1 shows that the protein is eluted at a size of 300 kD, twice the molecular weight of a FnCpf1 monomer (Figure S2B).

Zetsche et al, 2015. *Cell*, 163.

# **Results:** Use logical transitions between sections

…demonstrating that FnCpf1 and crRNA are sufficient for mediating DNA targeting (Figure 2c). By Contrast, Cas9 requires both crRNA and tracrRNA to mediate targeted DNA interference.

[New Section]
The finding that FnCpf1 can mediate DNA interference with crRNA alone is highly surprising given that Cas9 recognizes crRNA through the duplex structure between crRNA and tracrRNA, as well as the 3′ secondary structure of the tracrRNA. To ensure that crRNA is indeed sufficient for forming an active complex with FnCpf1…

Zetsche et al, 2015. *Cell*, 163.

# Results: There's a typical overall structure

In order to determine *X*, *Y* was performed, showing *Z* major results.

Data + conclusions

Options: pro, then con
most to least important
experiment vs. control

Transition

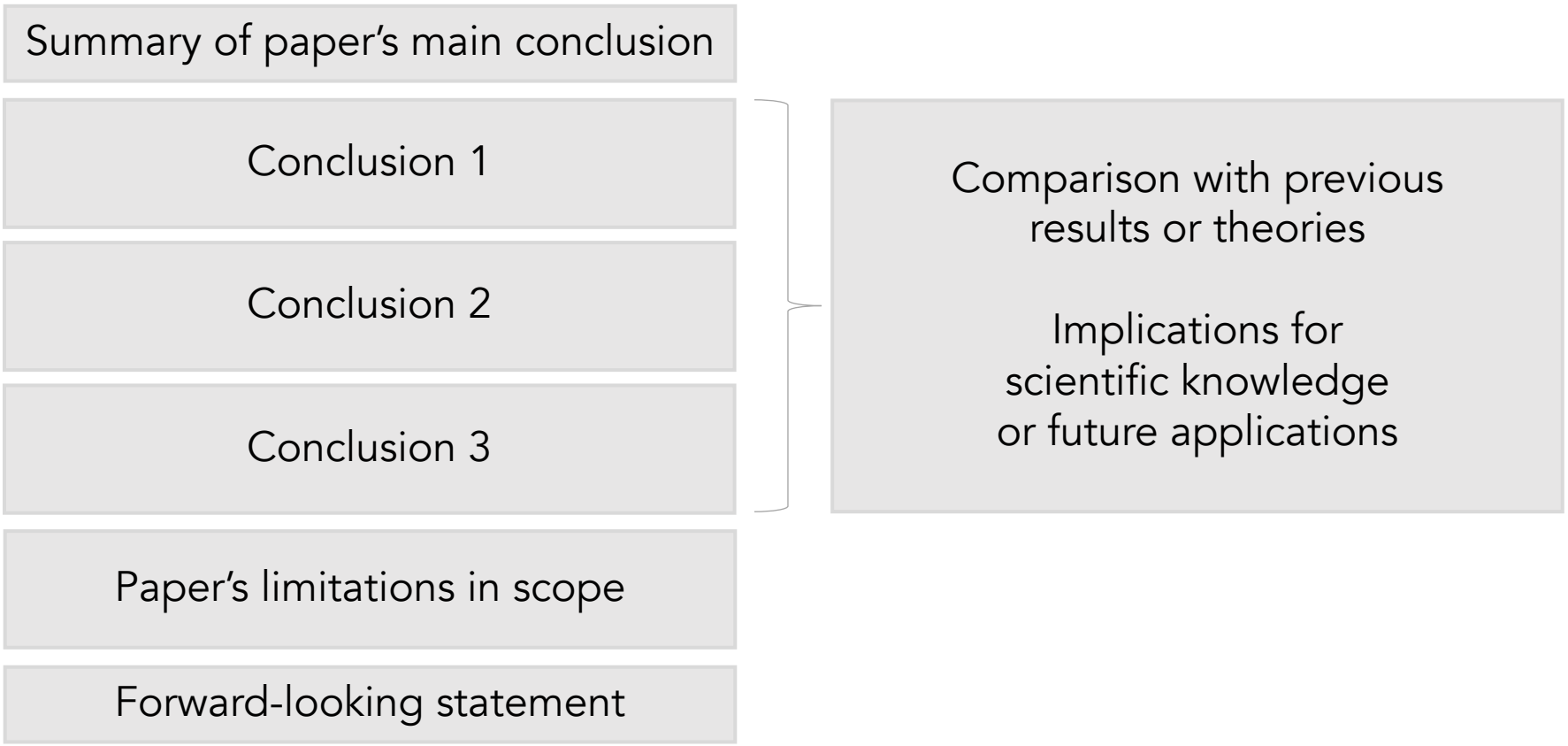
re-summarize findings
justify movement to next
experiment or hypothesis

The RuvC-like domain of Cpf1 retains all of the catalytic residues of this family of endonucleases (Figures 4A and S4) and is thus predicted to be an active nuclease. Therefore, we generated three mutants— FnCpf1(D917A), FnCpf1(E1006A), and FnCpf1(D1225A) (Figure 4A)—to test whether the conserved catalytic residues are essential for the nuclease activity of FnCpf1. We found that the D917A and E1006A mutations completely inactivated the DNA cleavage activity of FnCpf1, and D1255A significantly reduced nucleolytic activity (Figure 4B). These results are in contrast to the mutagenesis results for Streptococcus pyogenes Cas9 (SpCas9), where mutation of the RuvC (D10A) and HNH (N863A) nuclease domains converts SpCas9 into a DNA nickase (i.e., inactivation of each of the two nuclease domains abolished the cleavage of one of the DNA strands) (Jinek et al., 2012; Gasiunas et al., 2012) (Figure 4B). These findings suggest that the RuvC-like domain of FnCpf1 cleaves both strands of the target DNA, perhaps in a dimeric configuration. Interestingly, size-exclusion gel filtration of FnCpf1 shows that the protein is eluted at a size of 300 kD, twice the molecular weight of a FnCpf1 monomer (Figure S2B).

Zetsche et al, 2015. *Cell*, 163.

# Paper structure: Discussion

# Speculation and interpretation belongs in Discussion, not Results.



# The Discussion should start with a summary of the main message/conclusion

Summary of paper's main conclusion

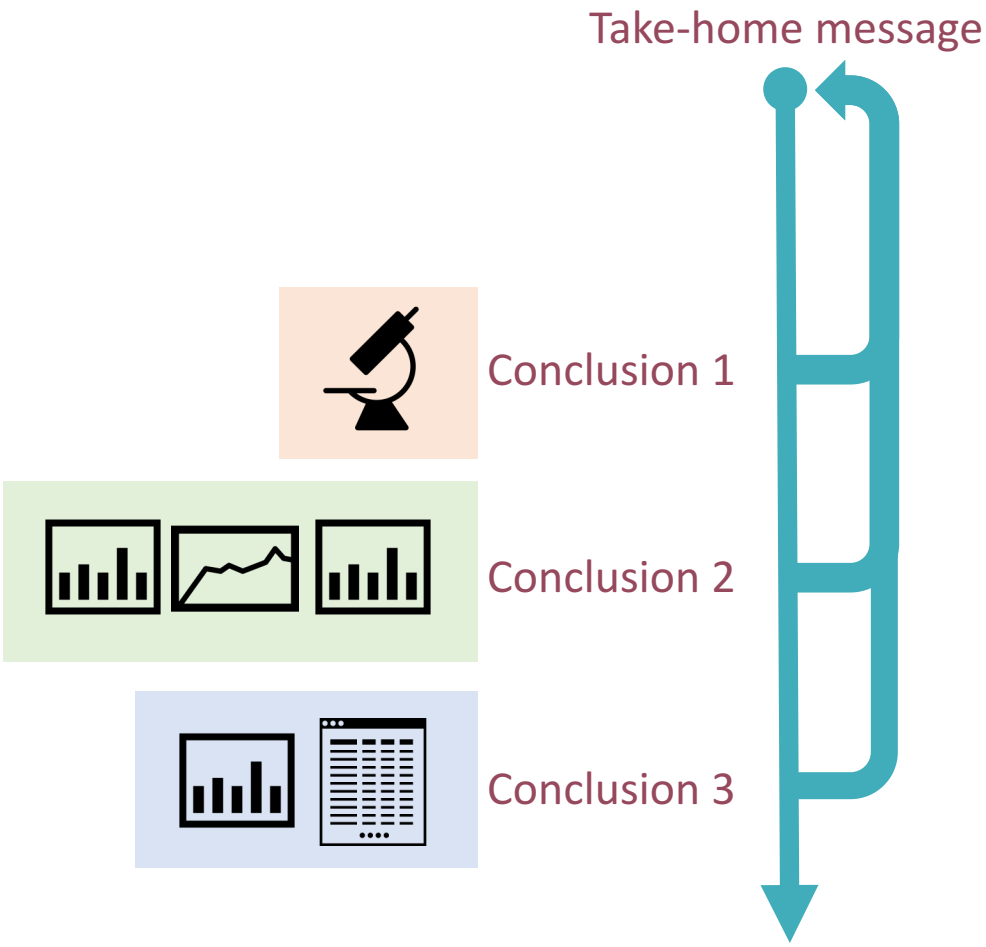
1 or 2 sentences

Reiterate your "here we show"

**DISCUSSION**

In this work, we characterize Cpf1-containing class 2 CRISPR systems, classified as type V, and show that its effector protein, Cpf1, is a single RNA-guided endonuclease. Cpf1 substantially

# Discussion: Discuss your main conclusions



# A successful Discussion answers questions for both experts and non-experts.

| | |
|---|---|
| Comparison with previous results or theories | *How do you account for results that contradict the rest of the field? How does it connect with other work?* |
| Scientific or engineering implications | *How will this work impact the field or people or the world?*<br><br>No more than 1 degree of speculation |
| Paper's limitations in scope | *How do you explain confusing or complicated results?* |

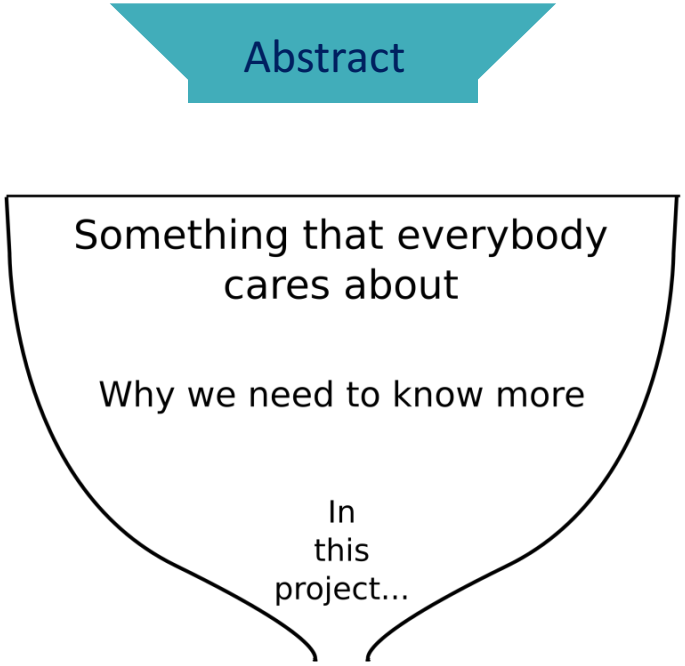
# Discussion often ends with a look at the future

The natural diversity of CRISPR systems provides a wealth of opportunities for understanding the origin and evolution of prokaryotic adaptive immunity, as well as for harnessing potentially transformative biotechnological tools. There is little doubt that, beyond the already classified and characterized diversity of the CRISPR-Cas types, there are additional systems with distinctive characteristics that await exploration and could further enhance genome editing and other areas of biotechnology as well as shed further light on the evolution of these defense systems.

# Paper structure: Introduction

# Introduction = Why did you do this research?

- Your research taught you something, right?
- Introduction convinces the reader that it's worth knowing
- background + knowledge gap + here we show



# Introduction: Clearly establish your central question and take-home message

- Clearly define the knowledge gap or central question of the study and follow with a clear hypothesis.
- Very briefly summarize the key results & conclusions of the paper.

**General background**
**Specific background**

**Knowledge gap,**
**Unknown**

**HERE WE SHOW**

**Results**
**Implication, Significance**

# Introduction: Clearly establish your central question and take-home message

Given the broad applications of Cas9 as a genome engineering tool (Hsu et al., 2014; Jiang and Marraffini, 2015), we sought to explore the function of Cpf1-based putative CRISPR systems.

Here, we show that Cpf1-containing CRISPR-Cas loci of Francisella novicida U112 encode functional defense systems capable of mediating plasmid interference in bacterial cells guided by the CRISPR spacers.

Zetsche et al, 2015. *Cell*, 163.

# Introduction: Briefly summarize key results

Unlike Cas9 systems, Cpf1-containing CRISPR systems have three features. **First,** Cpf1-associated CRISPR arrays are processed into mature crRNAs without the requirement of an additional trans-activating crRNA (tracrRNA) (Deltcheva et al., 2011; Chylinski et al., 2013). **Second,** Cpf1-crRNA complexes efficiently cleave target DNA proceeded by a short T-rich protospacer-adjacent motif (PAM), in contrast to the G-rich PAM following the target DNA for Cas9 systems. **Third,** Cpf1 introduces a staggered DNA doublestranded break with a 4 or 5-nt 50 overhang. **To explore the suitability of Cpf1 for genome-editing applications,** we characterized the RNA-guided DNA-targeting requirements for 16 Cpf1-family proteins from diverse bacteria, and we identified two Cpf1 enzymes from Acidaminococcus sp. BV3L6 and Lachnospiraceae bacterium ND2006 that are capable of mediating robust genome editing in human cells.

Zetsche et al, 2015. *Cell*, 163.

# Introduction: Identify the significance of your findings

Collectively, these results establish Cpf1 as a class 2 CRISPR-Cas system that includes an effective single RNA-guided endonuclease with distinct properties that has the potential to substantially advance our ability to manipulate eukaryotic genomes.

Zetsche et al, 2015. *Cell*, 163.

# Paper structure: Additional Tips

# Paragraph structure helps you and your reader

- One paragraph = one thought.
- 1st sentence summarizes this thought, last sentence reiterates.
- Elaborate in a logical order:
  - pro then con
  - most to least important evidence
  - chronological (be careful!)

# Pay attention to verb tenses

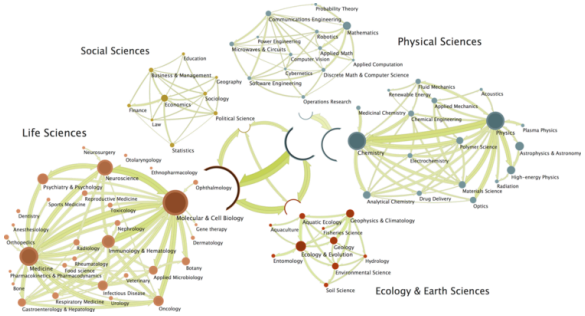
For information that is known or has previously been published: present tense

For information based on what you did that is new: past tense

Methods, Results: past tense

Abstract, Introduction, Discussion: tense switches

# References connect your paper to the research ecosystem




- Built over the course of the paper
- Make sure you include papers that…
  - reach conflicting conclusions
  - are from your competitors
  - were published during the course of your work

  (Reviewers will be looking)
- Your abstract will not have references, all other sections should!

# Revising is ESSENTIAL!

- Do not try to write this paper in one day.
- Outline/draft the sections, then set aside the paper for several days.
- If you get stuck: outline, write topic sentences, work on the next section, look at examples
- Get feedback:

  peers, instructors, Comm Lab Fellows!

# FAQ

- Figures and results on same page? Separate docs this time.
- You won't have a supplement
- Logical order of results: don't talk about Fig 5 in first result,
- Can you talk about things that happened earlier? Yes.
- Methods: in fig caption are conditions/controls, in results why you used conditions/controls, in methods exactly how

- Why are you doing expt, sentence before, we think this is...
- Conclusion to result motivates next expt
- But not inferences or interpretations save for discussion

# Assignment or paper questions?

20% of course grade (full rubric on wiki)

| | | |
|---|---|---|
| Title and Abstract | | 10% |
| Introduction | 2-3 p. | 10% |
| Methods | 3-4 p. | 20% |
| Results and Figures | 4-5 p. | 50% |
| Discussion | 2-3 p. | 10% |

(12pt., double-space except abstract, max. 14 pages)