Paper used as a case study today

Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression

https://tinyurl.com
/CRISPRi-20109

http://engineerbiology.org/w/images/2/24/Fa20_M3D1_CRISPRi_reference.pdf

Research manuscripts:

structure and writing process

20.109 Communication Workshop 5

Dr. Chiara Ricci-Tam

Dr. Sean Clarke

MIT BE BIOLOGICAL ENGINEERING | Communication Lab

Helping you communicate effectively.
mitcommlab.mit.edu/be/

The Broccoli and the Hourglass

Writing a paper integrates topics we have already covered…

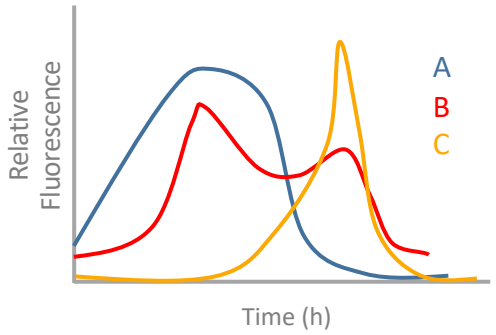
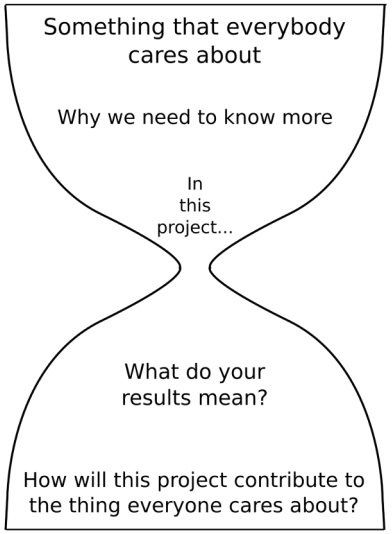


Fig. 1: A, B, and C have different dynamics under Condition X. A, B, and C were sampled using Method 1 and their fluorescence quantified with Method 2. Fluorescence data normalized to negative control.

Figures & Captions

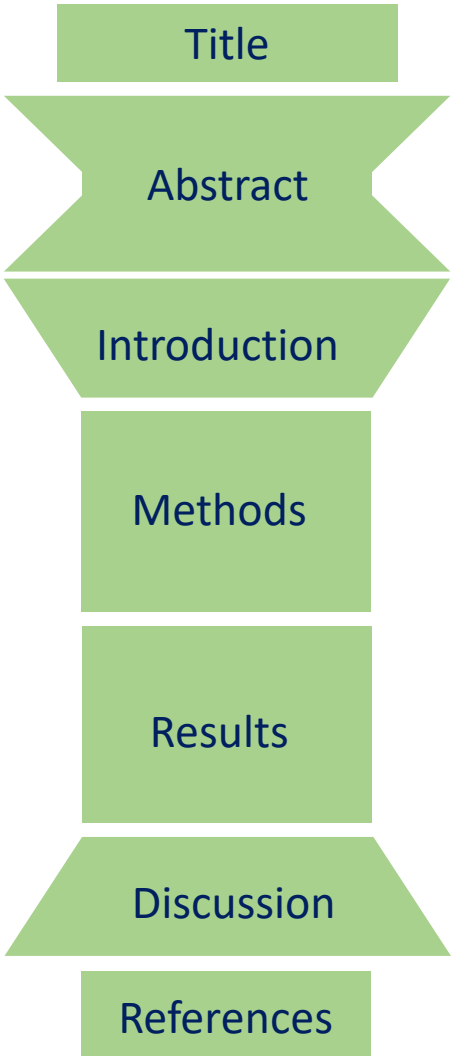
(Workshops 1 and 2)



Abstracts & Titles

(Workshops 2 and 3)

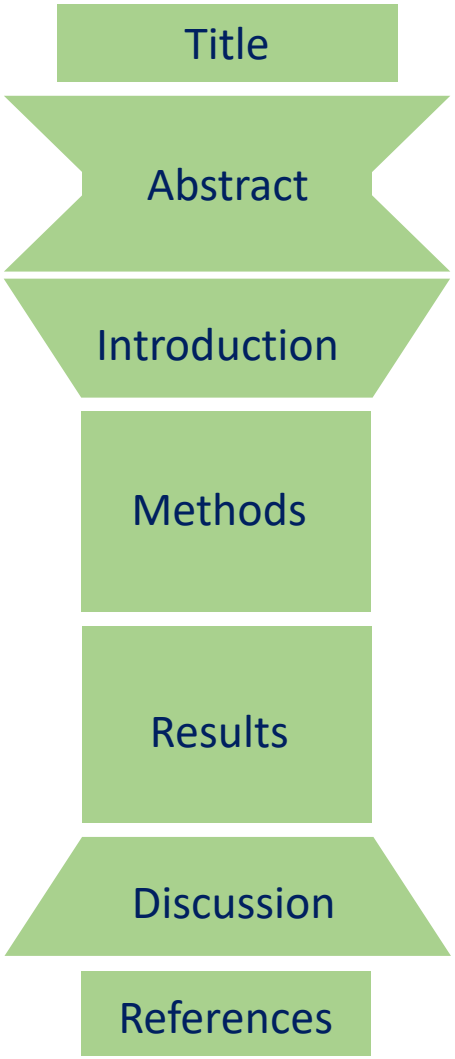
Discuss | In what order do you…



…**read** a paper?

…**write** a paper?

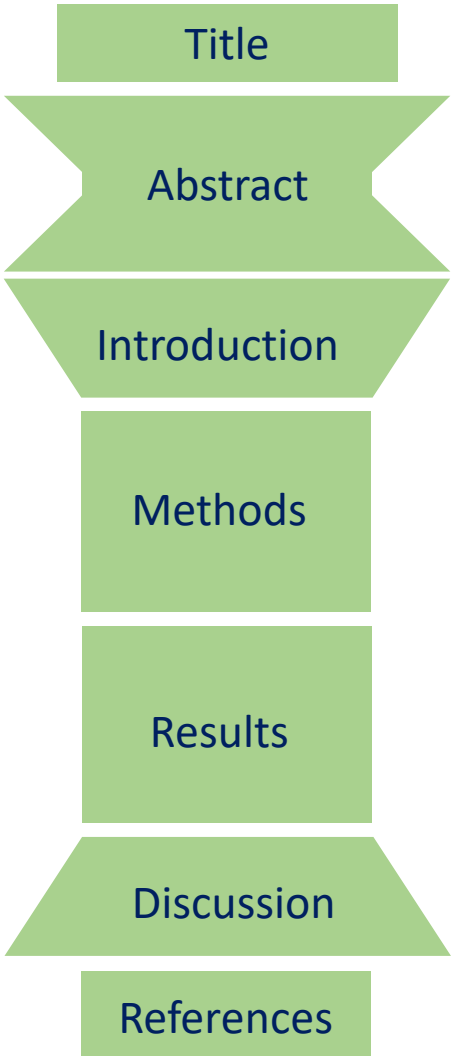
We recommend writing in this order



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

Methods?

We recommend writing in this order



1. Figures + Captions
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Methods?

http://dbis.rwth-aachen.de/~derntl/papers/misc/

There is more than one way to write a successful paper.

Collect papers that you like!

Analyze what makes them especially clear & compelling.

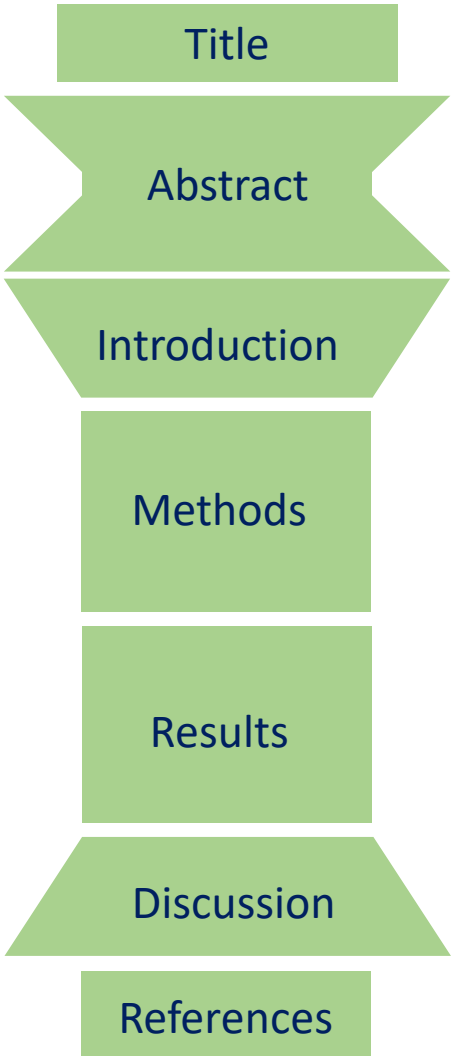
Try using their techniques.

What are characteristics of good writing?

clear and effective

elegant and stylish
(don’t worry about that now)

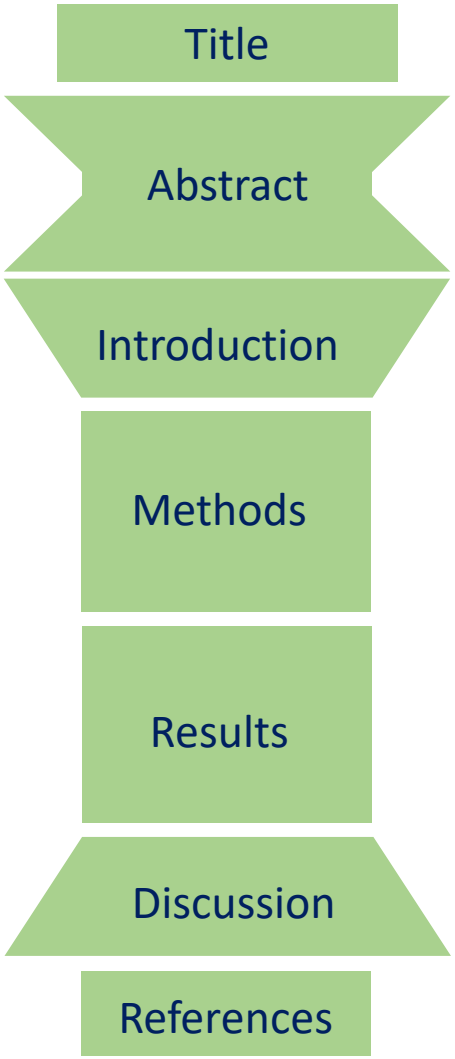
Clarity comes from **organization**



Sections guide us through the paper

Effective redundancy helps us to find information

Sections 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?

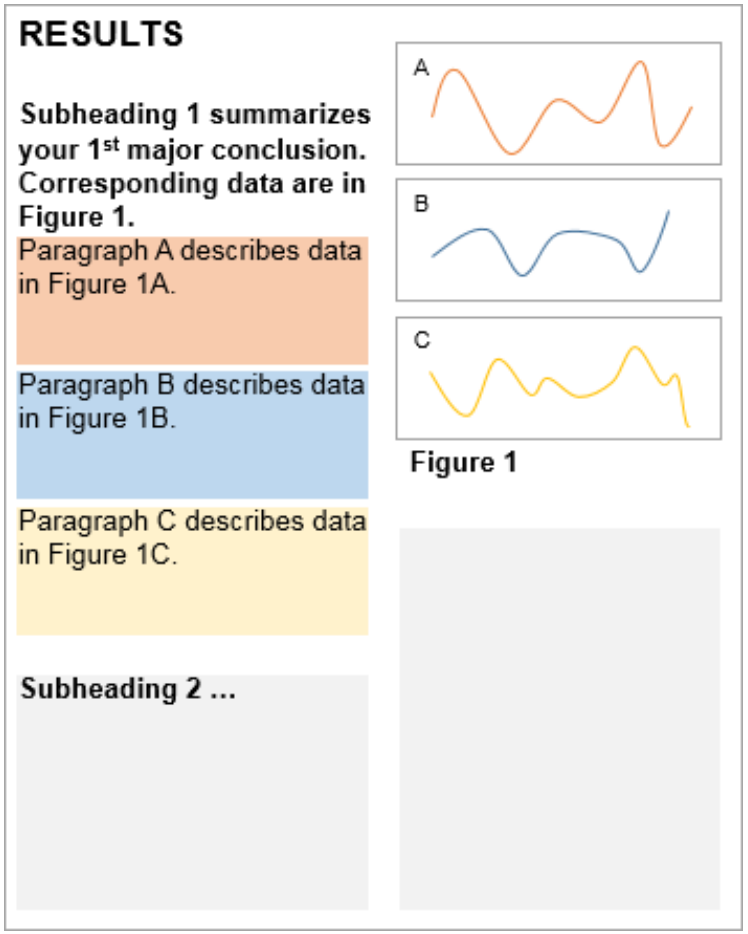
What other work is connected?

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 – beginning
Knowledge Gap / Unknown	Question that will be answered by your research; problem or phenomenon that is not understood	Introduction – end
Here We Show	Conclusion, answer to the unknown	Introduction – end Results – end Discussion – beginning
Results	Brief summary of approach + very high-level results. *Common pitfall = excessive detail*	Introduction (high level) Results Methods
Implication / Significance	So what? What do your results mean for the thing everyone cares about?	Discussion

Parallelism: content goes in same order

Data | Methods | Results | Discussion



Parallel organization, varying scope:

Methods: Most technical 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

1 thought, 1 paragraph

- Topic sentence summarizes this thought
- Last sentence reiterates and/or transitions
- Go in an order that's logical for the reader
  - pro then con
  - most to least important evidence
  - chronological (careful!)
- Organized paragraphs help you and your reader

CASE STUDY

Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression

http://engineerbiology.org/w/images/2/24/Fa20_M3D1_CRISPRi_reference.pdf

Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression

Targeted gene regulation on a genome-wide scale is a powerful strategy for interrogating, perturbing, and engineering cellular systems. **Here, we develop a method for controlling gene expression based on Cas9, an RNA-guided DNA endonuclease from a type II CRISPR system.** We show that a catalytically dead Cas9 lacking endonuclease activity, when coexpressed with a guide RNA, generates a DNA recognition complex that can specifically interfere with transcriptional elongation, RNA polymerase binding, or transcription factor binding. This system, which we call CRISPR interference (CRISPRi), can efficiently repress expression of targeted genes in Escherichia coli, with no detectable off-target effects. CRISPRi can be used to repress multiple target genes simultaneously, and its effects are reversible. We also show evidence that the system can be adapted for gene repression in mammalian cells. This RNA-guided DNA recognition platform provides a simple approach for selectively perturbing gene expression on a genome-wide scale.

Results

What did you find?

- You are stating what you did and what you found, not speculation
- You need to put your results in the order of your figures.

Results = rationale + data + conclusion

Conclusion 1

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 ...

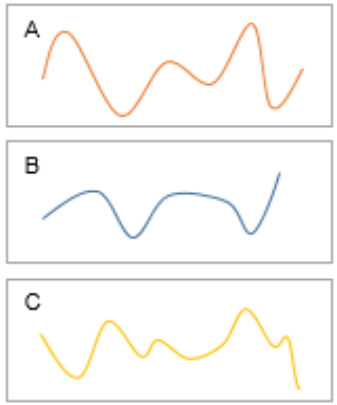


Figure 1

Results = rationale + data + conclusion

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

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: Present minimal essential data

Remember: MAXIMIZE signal-to-noise.

Include in your paper

- 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: Present minimal essential data

Remember: MAXIMIZE signal-to-noise.

Supplemental info

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: Keep a sense of proportion

space $\propto$ **importance**

spent describing an individual result

of that result to the paper's main conclusion

Ira Herskowitz, UCSF

Results: The heading of each result section reflects the message of that figure

Section title

A Minimal CRISPRi System Consists of a Single Protein and RNA and Can Effectively Silence Transcription Initiation and Elongation

Figure title

Figure 2. CRISPRi Effectively Silences Transcription Elongation and Initiation

Qi et al, Cell 2013

Results: Motivating the experiment

To test whether the dCas9:sgRNA complex could yield highly efficient repression of gene expression, we designed sgRNAs complementary to different regions of the mRFP coding sequence, either binding to the template DNA strand or to the nontemplate DNA strand. Our results indicated that sgRNAs targeting the nontemplate DNA strand demonstrated effective gene silencing (10- to 300-fold of repression), whereas those targeting the template strand showed little effect (Figure 2C).

Qi et al, Cell 2013

Results: Briefly what was done

To test whether the dCas9:sgRNA complex could yield highly efficient repression of gene expression, we designed sgRNAs complementary to different regions of the mRFP coding sequence, either binding to the template DNA strand or to the nontemplate DNA strand. Our results indicated that sgRNAs targeting the nontemplate DNA strand demonstrated effective gene silencing (10- to 300-fold of repression), whereas those targeting the template strand showed little effect (Figure 2C).

Qi et al, Cell 2013

Results: What was **FOUND** (data & conclusions)

To test whether the dCas9:sgRNA complex could yield highly efficient repression of gene expression, we designed sgRNAs complementary to different regions of the mRFP coding sequence, either binding to the template DNA strand or to the nontemplate DNA strand. Our results indicated that sgRNAs targeting the nontemplate DNA strand demonstrated effective gene silencing (10- to 300-fold of repression), whereas those targeting the template strand showed little effect (Figure 2C).

Qi et al, Cell 2013

Activity | *5min read + 2min discuss*

Glance through the results of the Lei et al 2013 Cell paper

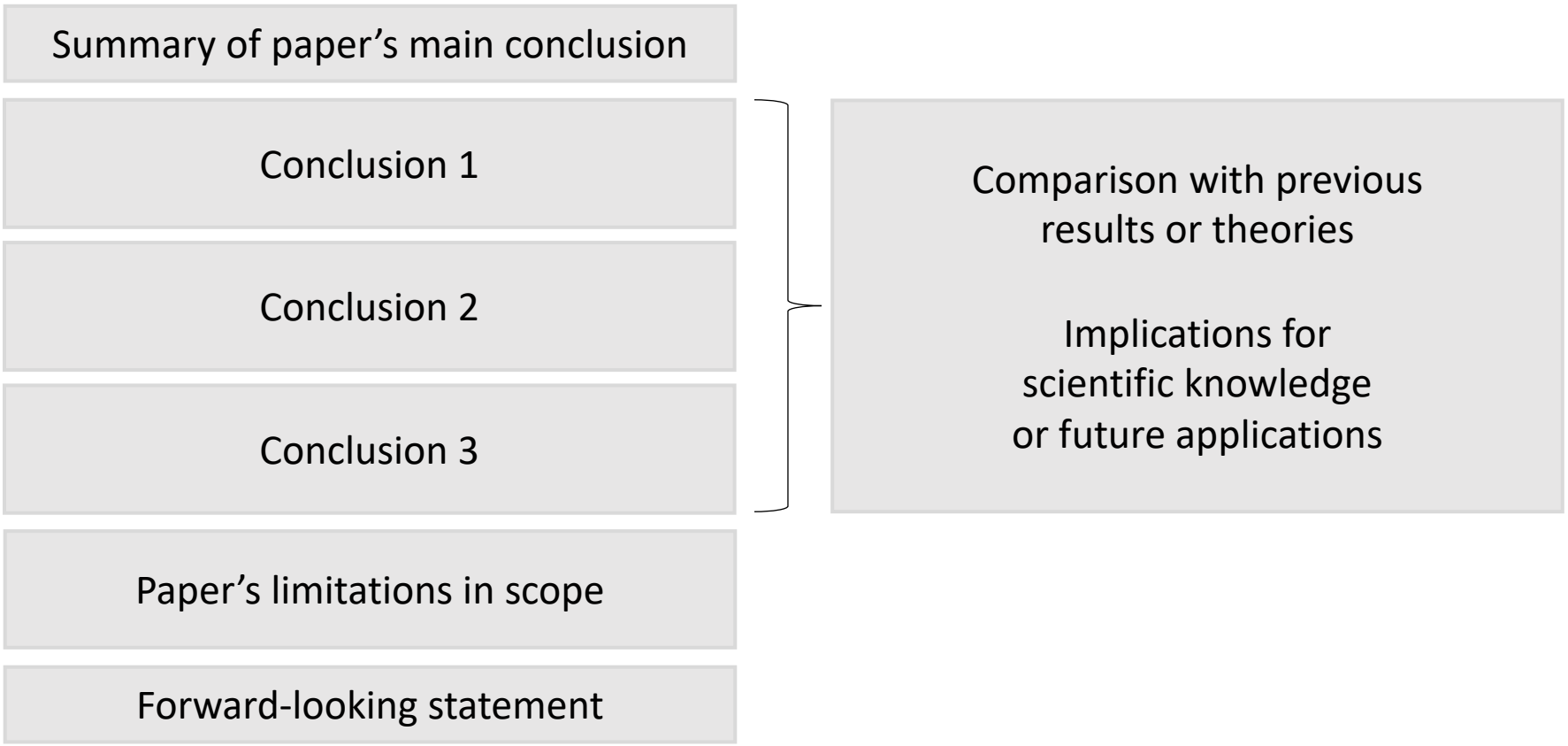
- Find places where you see “In order to find x, we did y, and we found z, which led us to our next experiment”
- If you don’t find this structure, how did the author introduce their results?
- What does the author NOT do in the results section?

Discussion

What does it all mean?

- Restate your main conclusion and findings
- Pick 2-4 points from your results that you want to discuss further (speculation allowed!)
- Restate the impact or future direction of your work

Speculation and interpretation belongs in the **Discussion**, not the 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"

The CRISPRi system that we report here is a relatively simple platform for targeted gene regulation. CRISPRi does not rely on the presence of complex host factors but instead only requires the dCas9 protein and guide RNAs and thus is flexible and highly designable.

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?* **No more than1 degree of speculation**
Paper's limitations in scope	*How do you explain confusing or complicated results?*

Discussion builds from the results

Comparisons?
Implications?
Limitations?

Particular phrases that would not be in other sections?

Differences in language?

CRISPRi Efficiently and Selectively Represses Transcription of Target Genes

Our results have demonstrated that the system can efficiently silence genes in bacteria. The silencing is very specific; we observe no detectable off-target effects. Furthermore, the efficiency of the knockdown can be tuned by changing the target loci and the degree of base pairing between the sgRNA and the target gene. This will make it possible to create allelic series of hypomorphs, a feature that will be especially useful for the study of essential genes. The system functions by directly blocking transcription in a manner that can be easily programmed by designing sgRNAs. To our knowledge, this is one of the first examples of utilizing a targeted proteinRNA complex that directly blocks transcription elongation within protein-coding regions. Mechanistically, this is distinct from RNAi-based silencing, which requires the destruction of already transcribed mRNAs.

Each paragraph can focus on the take home message of each figure

Summary of paper's main conclusion	
Conclusion 1	CRISPRi Efficiently and Selectively Represses Transcription of Target Genes
Conclusion 2	CRISPRi Is Amenable to Genome-Scale Analysis and Regulation
Conclusion 3	CRISPRi Provides Tools for Manipulating Microbial Genomes
Paper's limitations in scope	CRISPRi as a Platform for Engineering Transcriptional Regulatory Networks
Forward-looking statement	

Discussion often ends with a look at the future

Forward-looking statement

In summary, the CRISPRi system holds great promise as a general genetic programming platform that is suitable for a variety of biomedical research and clinical applications, including genome-scale functional profiling, microbial metabolic engineering, and cell reprogramming.

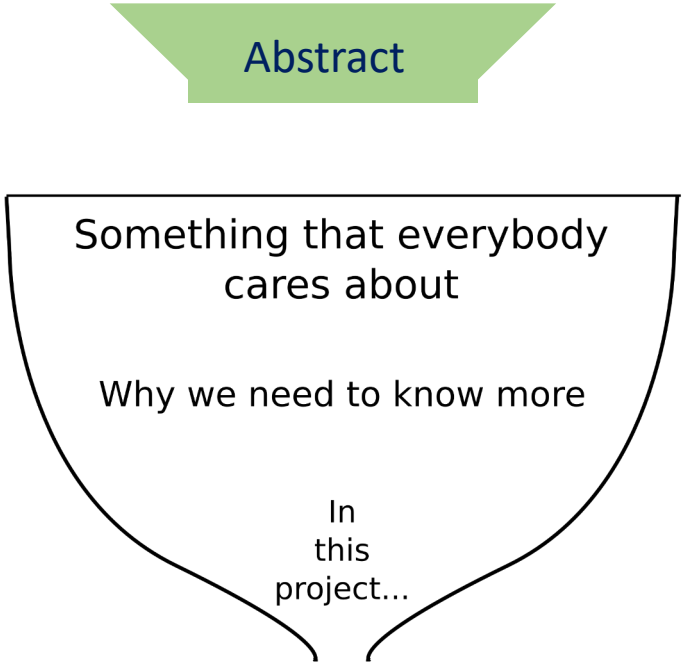
Introduction

What do I need to know to understand your story?

- Highlight key background information that your reader must know to understand your problem
- State your main conclusion and high level findings

Introduction = **Why** did you do this research?

- Explain the background for why you wanted to do this work
- Introduction convinces the reader that this knowledge is worth having
- background + knowledge gap + here we show



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

- Clearly define the knowledge gap/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

To repurpose the Cas9/CRISPR for genome regulation instead of genome editing, **here we demonstrate that** a catalytically inactive version of Cas9 can be repurposed as a platform for RNA-guided transcription regulation. The transcription of arbitrary genes can be modified by the mutant Cas9 without genetically altering the target sequence.

Introduction: Briefly summarize the key results

We show that CRISPRi silencing occurs by blocking transcription and is highly efficient with up to 1,000-fold repression. **We characterize** determinants of the regulatory efficiency, including target loci, length, and mismatches within the sgRNA base-pairing region. **We also show** that multiple sgRNAs can be used simultaneously to regulate multiple genes. **Furthermore, we demonstrate** that the CRISPRi system can be used to knock down endogenous genes and to profile cis regulatory elements for transcription factor binding in the lactose regulatory network. **Finally, we show** that the CRISPRi system can also be used to knock down gene expression in mammalian cells. The CRISPRi sequence-specific targeting platform **thus** holds promise as a general approach for modulating gene expression in a broad range of host cells.

Introduction: what should go in the background?

To repurpose the Cas9/CRISPR for genome regulation instead of genome editing, **here we demonstrate** that a catalytically inactive version of Cas9 can be repurposed as a platform for RNA-guided transcription regulation.

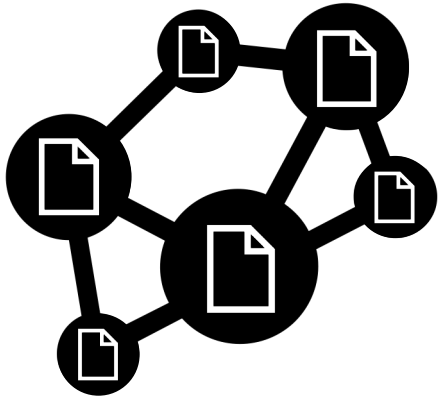
Introduction: provide minimal necessary context to appreciate main claim

To repurpose the Cas9/CRISPR for genome regulation instead of genome editing, **here we demonstrate** that a catalytically inactive version of Cas9 can be repurposed as a platform for RNA-guided transcription regulation.

Existing tools for genetic regulation
(e.g. interference as a genetic tool)

CRISPR to date

References connect your paper to the research ecosystem



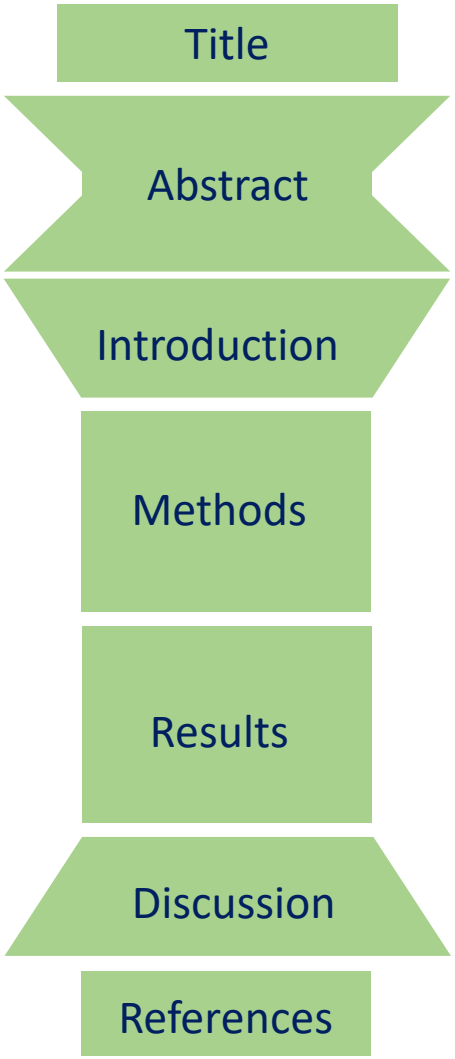
- Build them over the course of writing
- All sections except the abstract have references
- PRO TIP: include papers that…
  reach conflicting conclusions
  are from competitors
  were published during the course of your work
  (reviewers will be looking)

Revising is **essential**

- Do not try to write this paper all at once.
- **Outline, pause, draft**, set aside for a few days
- When you get stuck: write topic sentences, work on the next section, look at examples
- Get feedback:

  peers, instructors, Comm Lab Fellows!

Sections 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?

What other work is connected?

Any questions about assignment or papers in general?

Research article

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-spaced except abstract, max. 14 pages)