SURF PROPOSAL

INFO SESSION

Mark Conlan

Chris Bickford, PhD
STEM Writing Specialist
Lecturer in Engineering and Applied Science

HIXON WRITING CENTER

Goals of this session

- Break the SURF Proposal into manageable components
- Review a sample proposal to identify qualities of effective proposals
- Gain confidence about your (1) next steps and (2) ability to write a successful proposal

SURF Proposal Starter Kit

A Box link to the SURF Proposal Starter Kit will be emailed to everyone who fills out the linked form after the presentation concludes.

- SURF Proposal Starter
- SURF Proposal Overview and Reviewer Questions
- Annotated Mock BBE SURF Proposal (used in session)
- PDF of Info Session Slides
- Sign up instructions for meetings with Writing Specialists and tutors

ANNOTATED SURF PROPOSAL MODEL

1 The writer immediately situates the reader in a technical context by defining the subject of her research: the nucleosome. She decides the reader knows what chromatin, the genome, and DNA are. She also decides the reader understands how gene expression works in general. "Nucleosome" will be used throughout the proposal. The definition funnels into a BIG QUESTION.

2 The writer funnels the grand overture on nucleosomes, their importance in regulating gene expression, and the unknown in this specific domain into more specific background. Instead of writing more about what is known about how nucleosome positioning regulate gene expression, she decides to focus the current state of genome-wide nucleosome positioning studies. She narrows the scope even more by pinpointing the work done by the lab she plans to join. She chooses to write on this newly developed method to study nucleosome positioning because her objectives fall into this context. She bridges the 1st and 2nd paragraphs by using the consistent language. Specifically, the last 2 sentences of the first paragraph lead directly into the first sentence of the second paragraph.

Another paragraph on nucleosomes and how they regulate gene expression would've delayed the purpose of the proposed work and added unnecessary length to the proposal.

3 The writer saves the specifics of the method for later. She decides it's not important for the reader to know the specific details in the introduction. What is important is what this method has contributed to the field and how it relates to her proposed work.

HIGH-RESOLUTION NUCLEOSOME MAPPING IN MAMMALIAN CELLS

Sets up the general technical context for reader. Assumes the reader knows some basic molecular biology.

INTRODUCTION

1 The basic structural unit of eukaryotic chromatin is the nucleosome, which is composed of 147 base pairs of genomic DNA spooled around an octomeric histone core [1]. A majority of our genome is tightly bound by nucleosomes, which can occlude the underlying regulatory DNA sequences and limit their accessibility to regulatory factors that must act on them [2]. Consequently, where nucleosomes are positioned along DNA can determine the transcriptional output of the genome. However, the extent to which nucleosome positioning influences gene expression is unknown.

Greater scientific problem

2 Genome-wide nucleosome positioning has been most widely studied in yeast. Recently, the _____ lab developed a novel chemical mapping method to determine nucleosome positions in budding yeast *S. cerevisiae* [3,4]. This mapping method relies on site-directed hydroxyl radical cleavage of nucleosomes carrying modified histones to determine the positions of nucleosomes in the genome. The resultant map defined nucleosome positions at single base pair resolution and revealed new aspects of *in vivo* nucleosome organization for the entire yeast genome that had not been observed using previous mapping technology. Though general features of nucleosome dynamics might be shared between yeast and mammals, the size and complexity of the mammalian genome present a challenge to accurately mapping its nucleosomes. Such mapping would pave the way for better understanding the role nucleosomes play in gene regulation in higher organisms.

Status of related work by professor or group

More specific background

More specific problem and how it arose.

Why the solution is worthwhile

Asking Questions

- Feel free to raise your hand and ask questions at any point during the presentation

- Email bickford@caltech.edu

What is the SURF proposal?

The SURF proposal is

- A chance to better understand and prepare for your SURF project
- 1-3 page plan for your summer research:
  - What will you do?
  - How will you do it?
  - Why does it matter to science or society?

It should convince your reader

- You have a feasible project that you can accomplish or make significant progress on in 10 weeks

- Your proposed work is important and worthwhile

- You can write and think clearly as a scientist

- You're prepared to do the work you propose

The SURF proposal is <u>not</u>

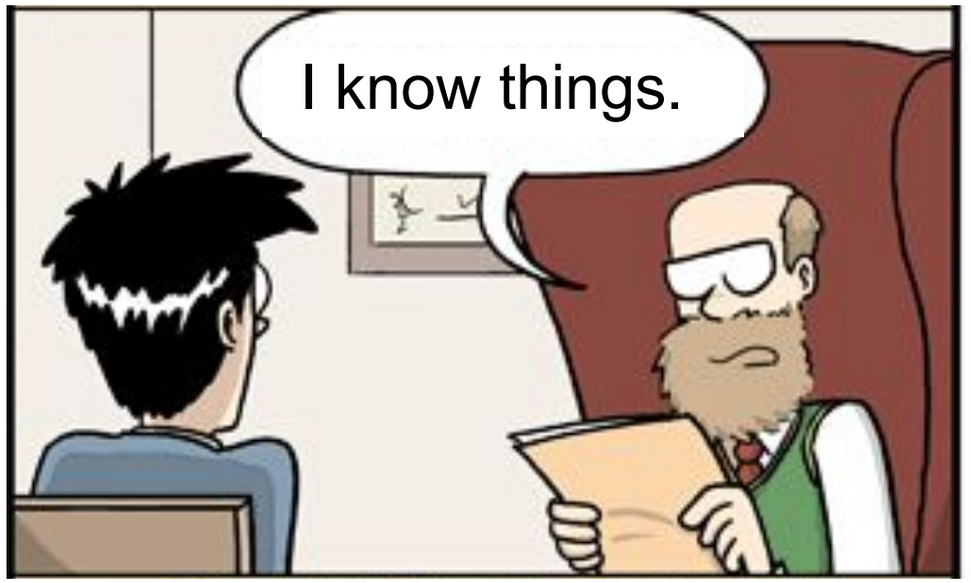
- A personal statement or a job application letter
- A research paper / article (no 2-column format)
- A place to discuss your previous research experiences in *other* labs/groups or your qualifications for SURF

Note: You <u>can</u> discuss results or progress you've made with the <u>same</u> group.

Who will read your proposal?

Mentor(s)

Knows your project inside and out



Other faculty members

Experts in the field (and, likely, in the big question you are studying) but not the nitty gritty details of *your* project

Recommended structure of a SURF Proposal

Title

Introduction

Objectives (or Aims)

Approach

Work Plan

References

Introduction/Background

- What is the background for your research?
- What is the challenge, gap, need, problem, etc?
- What is your solution?
- What is the significance of your work?

Tell a compelling story with the ABT structure

A B T

and

background and the significance

but

the conflict!
get here quick

therefore

what your proposal is all about:
proposed research

ABT is the DNA of Story

And

But

Therefore

INTRODUCTION

A The basic structural unit of eukaryotic chromatin is the nucleosome, which is composed of 147 base pairs of genomic DNA spooled around an octomeric histone core [1]. A majority of our genome is tightly bound by nucleosomes, which can occlude the underlying regulatory DNA sequences and limit their accessibility to regulatory factors that must act on them [2]. Consequently, where nucleosomes are positioned along DNA can determine the transcriptional output of the genome.

B However, the extent to which nucleosome positioning influences gene expression is unknown.

A

B

T

To date, three genome-wide nucleosome maps in mammalian cells have been reported [5,6,7]. However, due to their low-resolution, these maps are inadequate for studying the dynamic features of nucleosome positioning. To dramatically improve the resolution and accuracy of nucleosome mapping for mammalian cells, we propose to extend the chemical mapping method into mouse cells and construct the first high-resolution nucleosome map in the mammalian genome.

The first single base pair resolution mouse nucleosome map will advance our understanding of the dynamic interplay between nucleosome positioning and gene expression in higher organisms. Specifically, my SURF project will establish a genetic toolkit and a chemical mapping method that will allow researchers to interrogate the epigenomic function of nucleosomes in mammals.


Strong ABT =
Strong Story =
Strong Proposal

Avoid AAA and DHY

ABT = and, but, therefore

~~AAA = and, and, and~~ **never introduces problem**

~~DHY = despite, however, yet~~ **introduces too many problems**

Any questions at this point?

Objectives (Aims)

What do you plan to do in the 10 weeks of SURF?

Objectives

Frames the objectives in context of larger overarching goal, which cannot be achieved in 10 weeks.

Specific (achievable) aims are clearly delineated and not buried in a paragraph

The overall aim of this SURF project is to determine genome-wide nucleosome positions in mammalian cells at single base pair resolution. **The specific aims are to:**

(1) Engineer mutant H4S47C mouse embryonic stem (ES) (AB2.2 cell line) for chemical mapping.

Starting "knowns"

Uses clear verbs to describe work

(a) The chemical mapping approach requires introducing a unique cysteine into histone H4 at position 47 (H4S47C) to covalently attach a sulfhydryl-reactive copper-chelating label. This label enables the chemical cleavage of DNA at the nucleosome center. The criteria for success is our ability to show H4S47C mouse ES cells have sufficient levels of H4S47C and are functionally equivalent to wild type cells.

Writer identifies measure for success

(2) Establish and optimize the chemical mapping protocol for H4S47C mouse ES cells to demonstrate feasibility of chemically mapping nucleosomes in mammals.

(a) This protocol is based on the _____ lab's previously established protocol in yeast [4]. The success of this aim will be determined by our ability to (1) generate the desired cuts at nucleosome centers with limited non-specificity and (2) obtain sufficient amount of DNA for downstream analysis.

Starting conditions

Writer identifies measure for success

- Note: Aim 2 is dependent on the success of Aim 1.

Approach

What will you do in your 10 weeks of SURF in order to meet your objectives?

Help Your Reader Understand

Approach

We will analyze the sequences of 13 mouse H4 genes, identify two common regions shared by all H4 genes in mouse, and design shRNA constructs to knockdown all H4 genes in the mouse genome. Concurrently, we will synthesize an RNAi-resistant H4S47C transgene vector that will express the H4S47C transgene in the mouse ES cells.

TO UNDERSTAND

Methods

To construct mU6-driven H4-shRNA, the oligos will be phosphorylated, annealed, and ligated into a BbsI and XhoI digested *PB-mU6::PGK-Puro* vector: H4-Sh1 sense and H4-Sh1 antisense, H4-Sh2 and H4-Sh2, and H4-Sh3 sense and H4-Sh3 antisense to target all mouse H4 genes. To express H4S47C in the presence of H4-shRNA, we will synthesize a codon-modified, RNAi-resistant H4S47C cDNA expression vector *PB-CAG-H4S47C::PGK-Hygro*.

TO BORROW

Protocol

I will construct mU6-driven H4-shRNA. First, I will set up the following reaction in a microcentrifuge tube on ice:

COMPONENT	20 µl REACTION
T4 DNA Ligase Buffer (10X)*	2 µl
BbsI/XhoI cut PB-mU6::PGK-Puro vector	50 ng (0.020 pmol)
H4-shRNA 1	37.5 ng (0.060 pmol)
Nuclease-free water	to 20 µl
T4 DNA Ligase	1 µl

(T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.)

TO REPLICATE

The chemical mapping strategy relies on the substitution of wild type histone H4 with H4S47C, in which serine 47 has been replaced with a cysteine [3,4]. The unique cysteine in H4S47C symmetrically flanks the nucleosome center axis and is in close proximity to the DNA backbone (Fig. 1A). Covalent linkage of a sulphydryl binding copper-chelating label (phenanthroline-iodoacetamide) to the cysteine anchors a copper ion to the DNA at the same position— symmetric around the center axis (Fig. 1A). With the addition of hydrogen peroxide, the copper becomes a site of hydroxyl radical production, and a localized hydroxyl radical reaction cleaves the DNA precisely at the center (Fig. 1B). H4S47C-targeted nucleosome cleavage generates a characteristic DNA ladder in the presence of the copper chelator (Fig. 1C). Each step in the DNA ladder in the agarose gel represents the center-to-center distance between two adjacent nucleosomes.

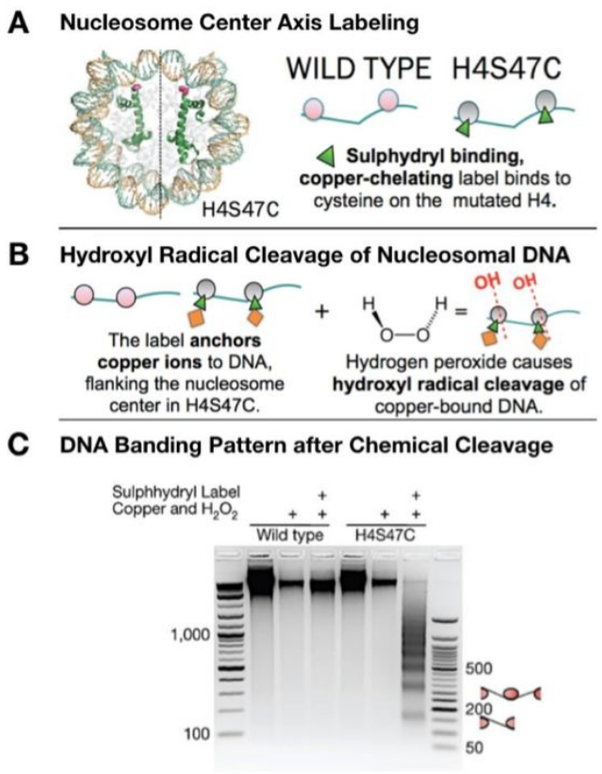


Fig 1. The chemical mapping method. (A) Histone core contains a modified H4 protein where serine 47 has been mutated to a cysteine, symmetrically flanking nucleosome center axis . (B) Cleavage of copper-bound DNA is specific to center axis. (C) Agarose gel showing the chemical mapping results in a DNA band pattern, which occurs only when the reaction includes the sulphydryl-reactive label, copper, H_2O_2, and H4S47C mutant.

Specific Aim 1: The first step in developing a chemical mapping strategy for mouse ES cells is to substitute multiple endogenous histone H4 with H4S47C. The mouse genome encodes 13 histone H4 genes, each of which encodes for identical H4 proteins. However, it is impossible to replace all 26 alleles of mouse H4 genes with the engineered H4S47C through gene targeting. Chemical mapping experiments in fission yeast *S. pombe*, however, showed that substitution of only two of the three H4 genes with H4S47C produced comparable levels of chemical cleavage to the strain with all three H4 genes replaced [8]. Therefore, we will design a strategy to replace a majority of endogenous H4 proteins with mutant H4S47C by a combination of RNAi knockdown and cDNA expression.

To determine whether chemical mapping strategy is feasible in mouse ES cells, we will analyze the sequences of 13 mouse H4 genes, identify two common regions shared by all H4 genes in mouse, and design shRNA constructs to knockdown all H4 genes in the mouse genome (Fig. 2A, box). Concurrently, we will synthesize an RNAi-resistant H4S47C transgene vector that will express the H4S47C transgene in the mouse ES cells.

Lays the foundation for understanding the methodology; provides a figure to help reader visualize what would be too complex to understand without it

Lays out a principal step

Writer identifies challenge in this step

Writer cites idea/ inspiration from outside related work as a step toward overcoming challenge

Writer provides narrative for understanding next steps

To test whether H4-shRNA constructs can efficiently target endogenous wild type H4 and not the synthetic H4S47C, we will transiently transfect each H4-shRNA with Flag-tagged-H4 and Flag-tagged H4S47C into HEK 293T cells and test for loss of Flag-tagged-H4 protein expression by Western blot analysis. After confirming that our shRNA constructs are specific only for endogenous H4, we will use a PiggyBac transgenic approach to simultaneously express the H4-shRNA and the H4S47C into mouse ES cells (A. Fox, Personal Communication, Dec 30, 2013). After sequential drug selection with Hygromycin (for PB-CAG-H4S47C vector) and Puromycin (for U6-shRNA-H4 vector), we will establish several stable ES cell clones (Fig. 2) and analyze the clones for functional equivalence to wild type cells. First, we plan to analyze expression levels of synthesized H4S47C and endogenous H4 by RT-PCR and select the cell lines with high levels of H4S47C and low levels of endogenous H4 for further validation and characterization. The criteria for selection include choosing mutant H4S47C ES cells with total H4 protein levels comparable to levels in the wild type ES cells. Second, we will select H4S47C-expressing ES cells that demonstrate similar growth patterns to wild type cells and express comparable levels of core pluripotency protein factors Oct4, Nanog, and Sox2.

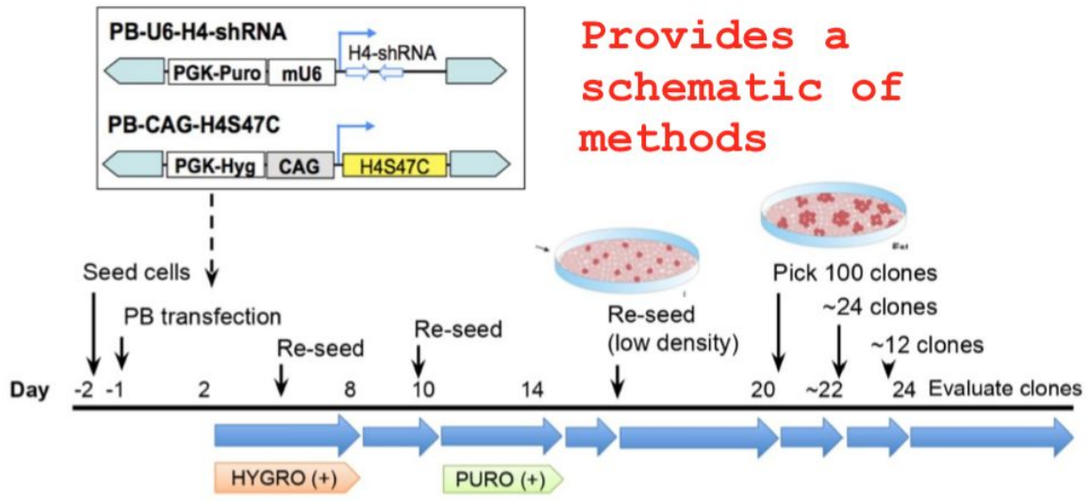


Fig 2. Timeline for generating H4S47C mouse ES cells. Sequential drug selection with Hygromycin (for PB-CAG-H4S47C) and Puromycin (for U6-shRNA-H4) to establish stable ES cell clones. The box shows the PiggyBac vectors for the H4-shRNA and H4S47C transgene.

Toward **Specific Aim 2**, we plan to adapt the chemical mapping protocol to the engineered H4S47C cells. This protocol is based on the previously established protocol in *S. cerevisiae* [3,4]. We will need to make significant modifications in cell permeabilization methods and the concentrations and incubation times with the copper-chelating label. By optimizing these conditions, we aim to show by DNA agarose gel that H4S47C-targeted nucleosome cleavage in ES cells can generate characteristic DNA ladder in the presence of the copper chelator. Following the mapping protocol, we will use the Qiagen PB system to purify the DNA fragments representing the center-to-center distance between two adjacent nucleosomes and quantify the sample by NanoDrop.

Provides a schematic of methods

Writer gives details on any established procedures

Provides details (names) of important materials or techniques used

Provides criteria for moving forward to next objective/aim

Cites any work that precedes it and lays foundation for next approach

Materials and Equipment used

Work Plan: establish the timeline

What week(s) will you do what you said you'd do in the approach section?

Work Plan

If you are performing multiple tasks <u>sequentially</u>:

Pre-SURF - Week 1 | (1) Design RNAi-resistant H4S47C transgene vector and shRNA constructs for H4 knockdown (2) Prepare and expand mouse ES cell cultures

Week 2 - 4 | Sequential drug selection with Hygromycin (for PB-CAG-H4S47C) and Puromycin (for U6-shRNA-H4) to establish stable ES cell clones.

Week 5 - 7 | Evaluate clones via functional assays: RT-PCR, Western blot, and growth curves to test for normal cell behavior and gene/protein expression in mutant ES cell clones.

Week 8 - 10 | Optimize chemical mapping protocol for mouse ES cells and purify nucleosomal DNA

Writer organizes major activities and demonstrates (in a perfect world) the project can be completed in 10 weeks

This work plan calls back what was discussed in the approach.

Work Plan

If you are performing multiple tasks <u>in parallel</u>:

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Task 1										
Task 2										
Task 3										
Task 4										
Task 5										

Work Cited / References

What references can you bring in to support your ideas, definitions, and plans and to demonstrate your knowledge of the field/relevant literature?

Work Cited / References

- Choose a scientific citation style (*ACS*, *Nature, Science*)
- Be consistent
- Use in-text citations (not just bibliography)
- More references = stronger proposal
- Consider learning to use a citation management system (Zotero, Endnote, Mendeley, Cite This For Me)

Work Cited / References

[1] K. Luger *et al.*,, Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* 389, 251 (1997).

[2] T. J. Richmond, C. A. Davey, The structure of DNA in the nucleosome core. *Nature* 423, 145 (2003).

[3] A. Flaus, T. J. Richmond, Base-pair resolution mapping of nucleosome positions using site- directed hydroxy radicals. *Methods Enzymol* 304, 251 (1999).

[4] K. R. Brogaard *et al.*, A chemical approach to mapping nucleosomes at base pair resolution in yeast. *Methods Enzymol* 513, 315 (2012).

[5] D. E. Schones et al., Dynamic regulation of nucleosome positioning in the human genome. Cell 132, 887 (2008).

[6] A. Valouev et al., Determinants of nucleosome organization in primary human cells. Nature 474, 516 (2011).

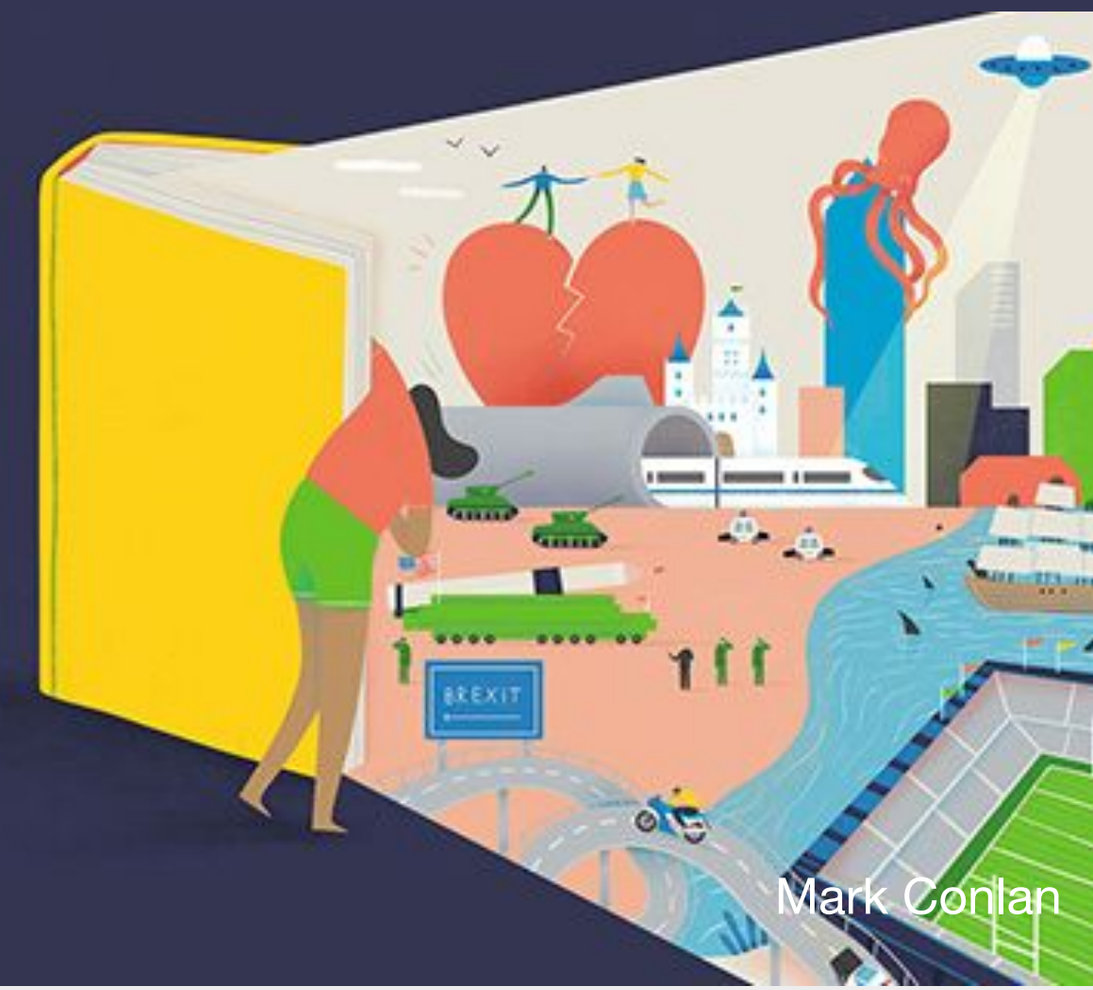
[7] V. B. Teif et al., Genome-wide nucleosome positioning during embryonic stem cell development. Nat Struct Mol Biol 19, 1185 (2012).

[8] G. Moyle-Heyrman *et al.*, Chemical map of Schizosaccharomyces pombe reveals species-specific features in nucleosome positioning. *Proc. Natl. Acad. Sci.* 110 (2013).

Before You Start Writing

- **Discuss project with mentor**
  Set up a meeting with grad student/post-doc/staff mentor and, if possible, with the faculty (PI) mentor

- **READ, READ, READ!**
  Published papers
  Previous proposals
  Internal documents

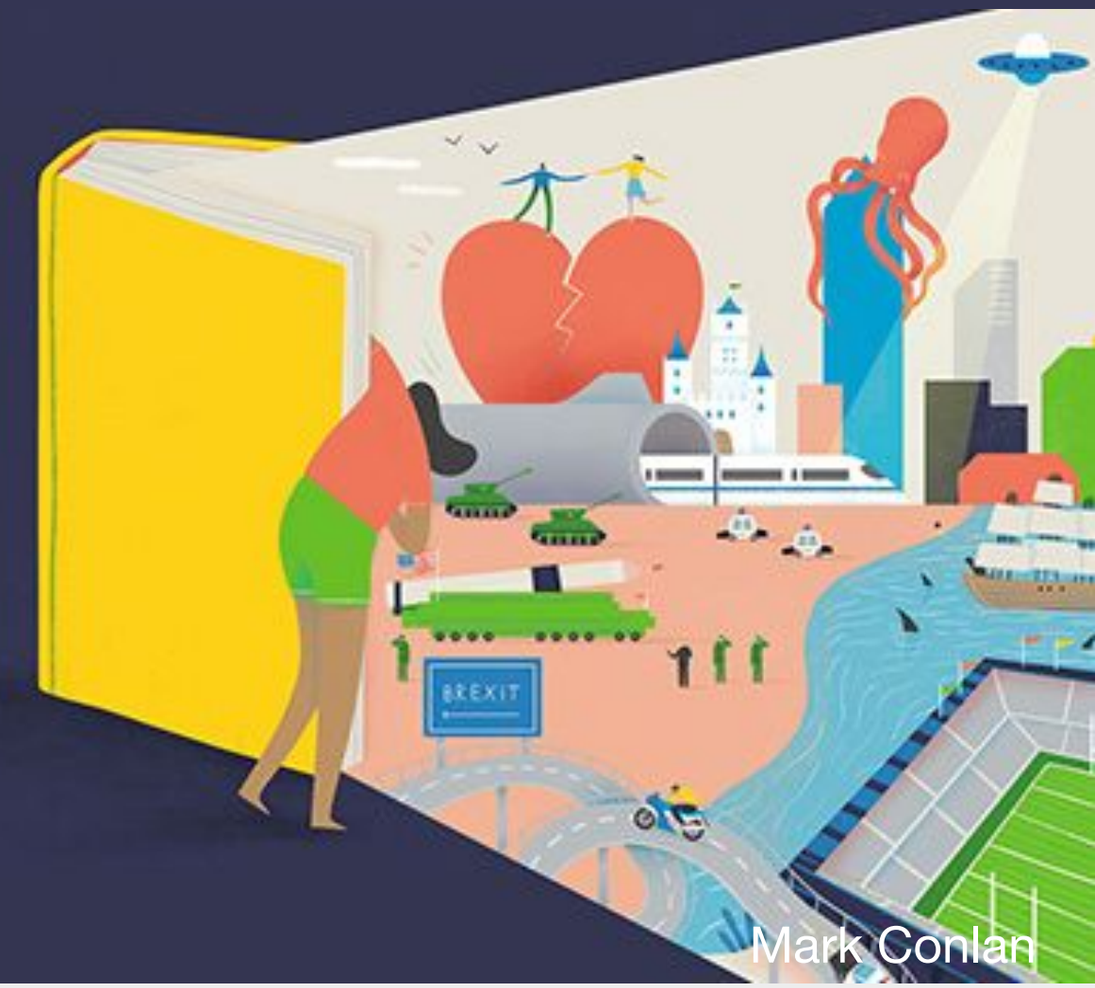


Mark Conlan

Key Questions to Consider

Example questions for mentor:

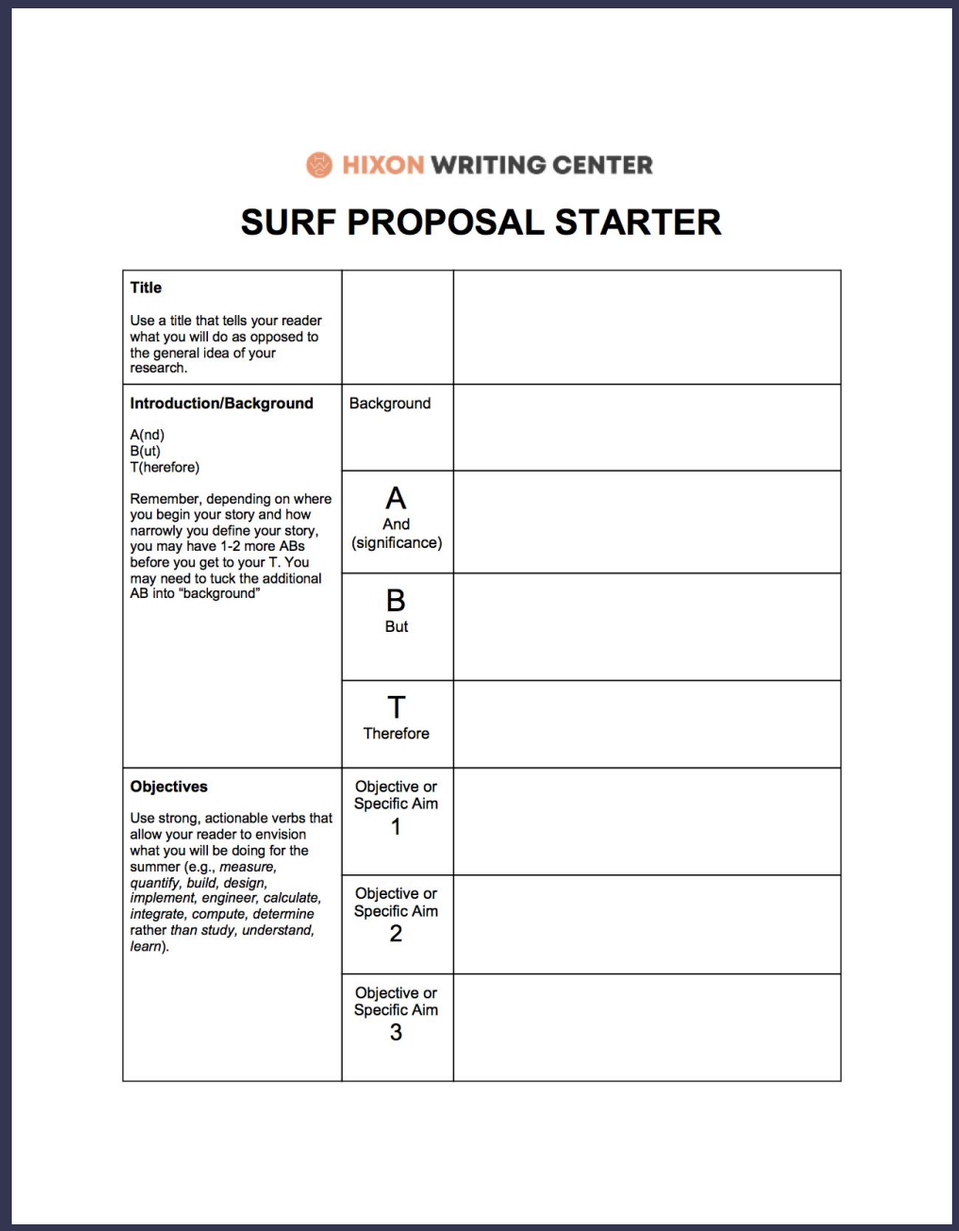
- What is the big picture?
- Specific objectives?
- How much flexibility or independence in designing the research, experiments, testing, etc?
- Where are we in the project?
- Technical hurdles to overcome in the experiment, test, design, etc?
- Mentor's role and involvement?



Mark Conlan

HWC SURF Proposal Starter

- **Draft an outline / fill out the starter** with specifics (as much as you can)
- **Find out what you don't know**
- **Bring to research mentor or HWC Writing Specialist or Peer Tutor to discuss**



HIXON WRITING CENTER

SURF PROPOSAL STARTER

Title Use a title that tells your reader what you will do as opposed to the general idea of your research.		
Introduction/Background A(nd) B(ut) T(herefore) Remember, depending on where you begin your story and how narrowly you define your story, you may have 1-2 more ABs before you get to your T. You may need to tuck the additional AB into "background"	Background	
	A And (significance)	
	B But	
	T Therefore	
Objectives Use strong, actionable verbs that allow your reader to envision what you will be doing for the summer (e.g., *measure, quantify, build, design, implement, engineer, calculate, integrate, compute, determine* rather *than study, understand, learn*).	Objective or Specific Aim 1	
	Objective or Specific Aim 2	
	Objective or Specific Aim 3	

Mark Conlan

Get help with your SURF Proposal at the Writing Center

- One-to-one meetings with Writing Specialists or peer tutors
- Come at *any stage* of the writing process
- View samples of successful SURF proposals
- Virtual or in-person meetings
- Located 3rd floor Center for Student Services

How to Sign Up

Visit access.caltech.edu >

Writing Center Scheduling >

book open times

In case you missed it earlier, this QR links to a form that will ask for your Caltech email address. We will send you a Box link to a SURF proposal starter kit.

Need more information?
Contact us at writing@caltech.edu

Thank You For Attending!

HIXON WRITING CENTER