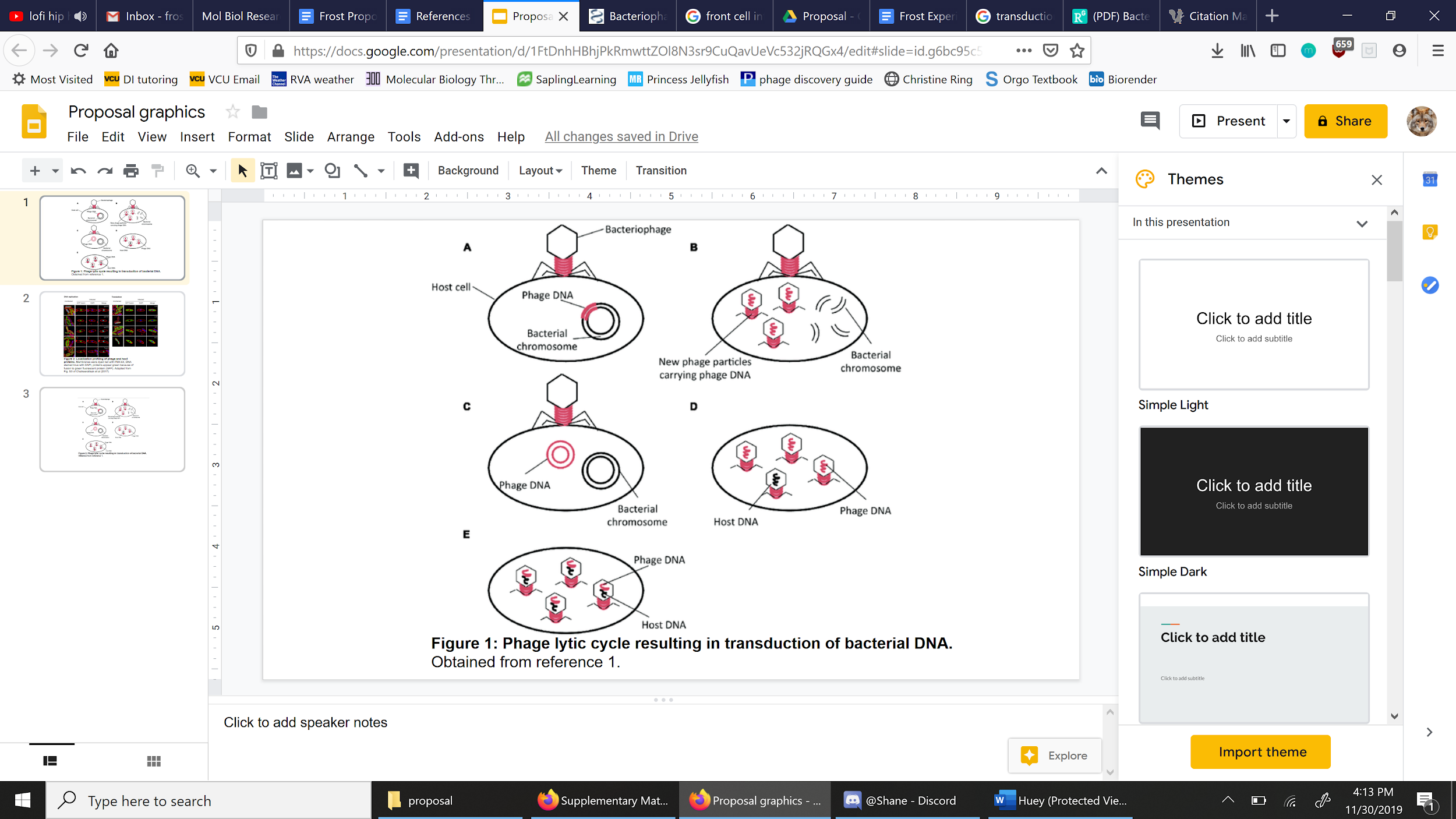
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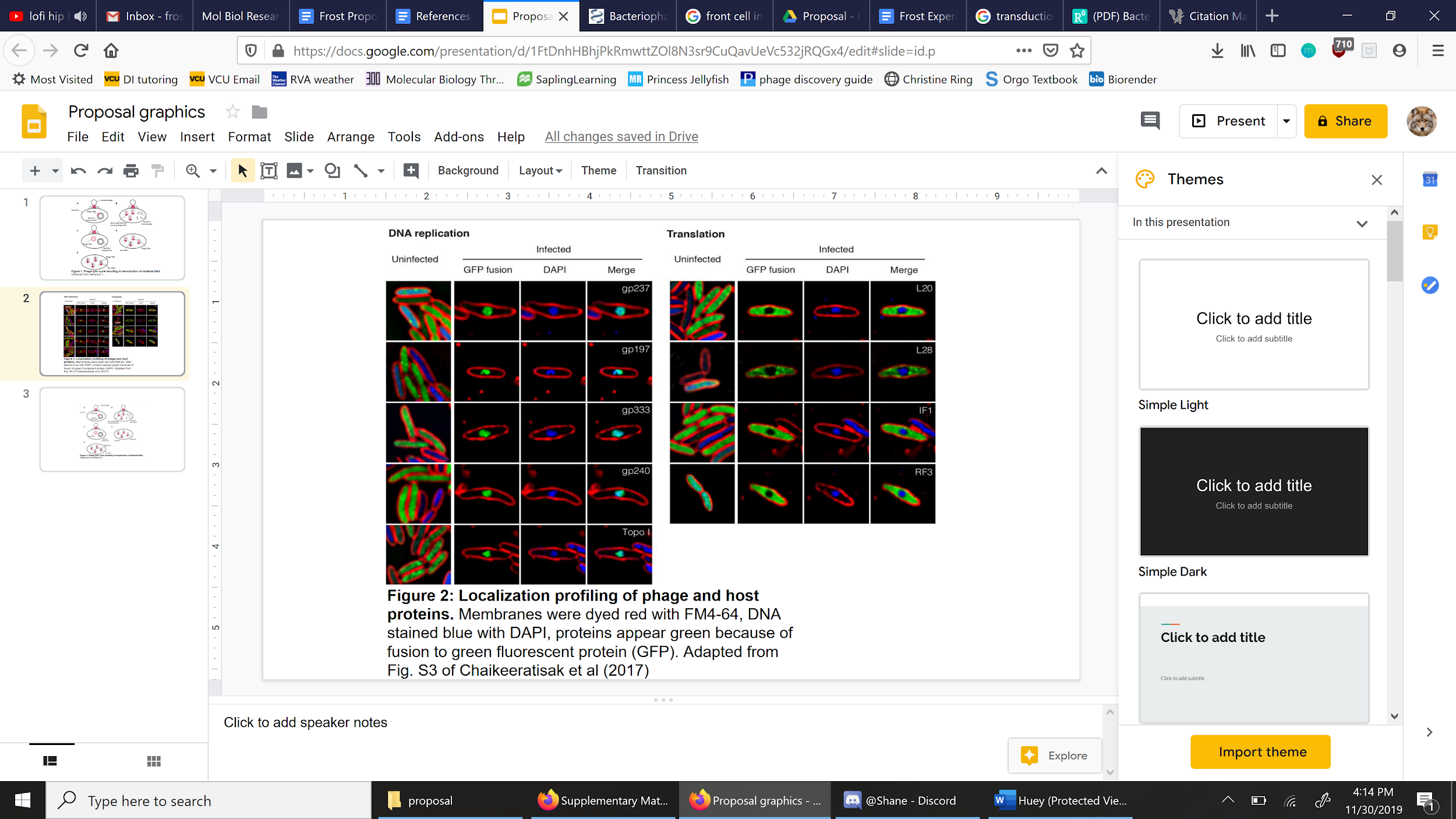
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Endeavoring to Find the Mechanism by which *Pseudomonas chlororaphis* Phage 201ϕ2-1 Proteins are Sorted within the Phage-Formed Proteinaceous Shell

**I. Introduction**

Bacteriophages (“phages”) are viruses evolved to target only bacteria. Individual phage are highly specialized, attacking only a small range of bacterial species. They are almost inconceivably diverse, with an estimated population of 1031 individuals on Earth. Phages are deeply intertwined with their hosts’ evolution, being both a predatory force they need to out-evolve and a vector for horizontal gene transfer.[[1]](#footnote-0) There’s even evidence that some bacteria will only become pathogenic when a phage able to code for the correct toxins is “lysogenized” into the host’s genome.[[2]](#footnote-1) Because they are so ubiquitous, it is crucial to study phages in order to form a more accurate understanding of the complex microbiomes all around us. With a better understanding of bacteria and their viruses, scientists will be able to work more effectively to create new products and medicines for the benefit of humanity.

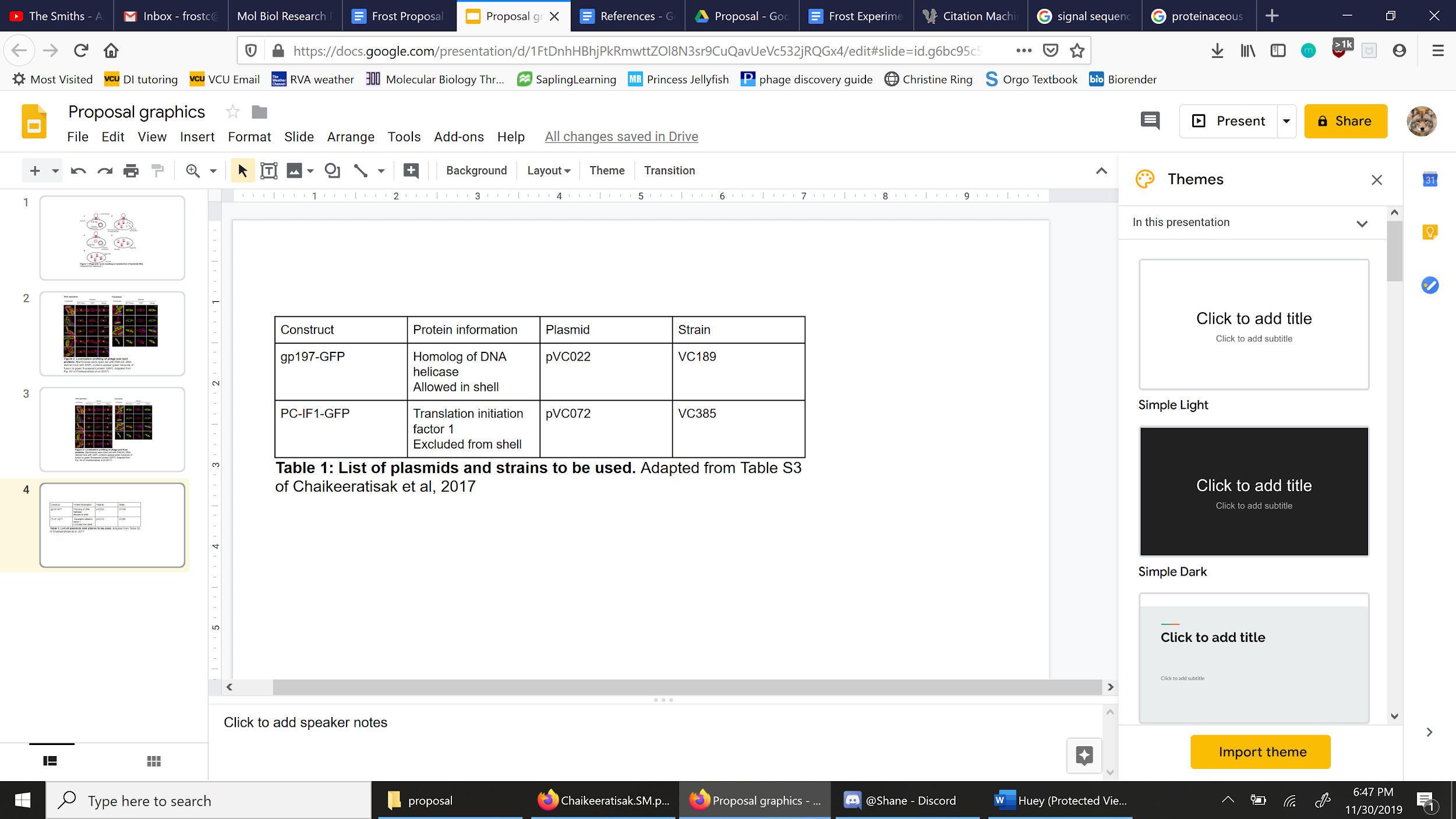
Phage generally hijack their host’s reproductive machinery to create new copies of themselves in the cytoplasm of the host. However, *Pseudomonas chlororaphis* (*P. chlororaphis*) phage 201ϕ2-1 (alternately written as 201𝜑2-1 or 201phi2-1) was recently identified as forming a unique protein shell within its host that was centered by cytoskeletal proteins (Chaikeeratisak et al, 2017). Fusions between green fluorescent protein (GFP) and gp105 (a highly expressed protein generated by 201phi2-1’s infection[[3]](#footnote-2)) were created and observed in the cell using localization profiling. As infection progressed, gp105-GFP was shown to form a small focus at one of the cell’s poles before being moved to the midcell by cytoskeletal protein fusion mCherry-PhuZ where the mass continued to grow (Chaikeeratisak et al, 2017). All cells infected by 201phi2-1 had at least one of these nucleus-like structures, with about 13% of infected cells containing two or more (Chaikeeratisak et al, 2017).

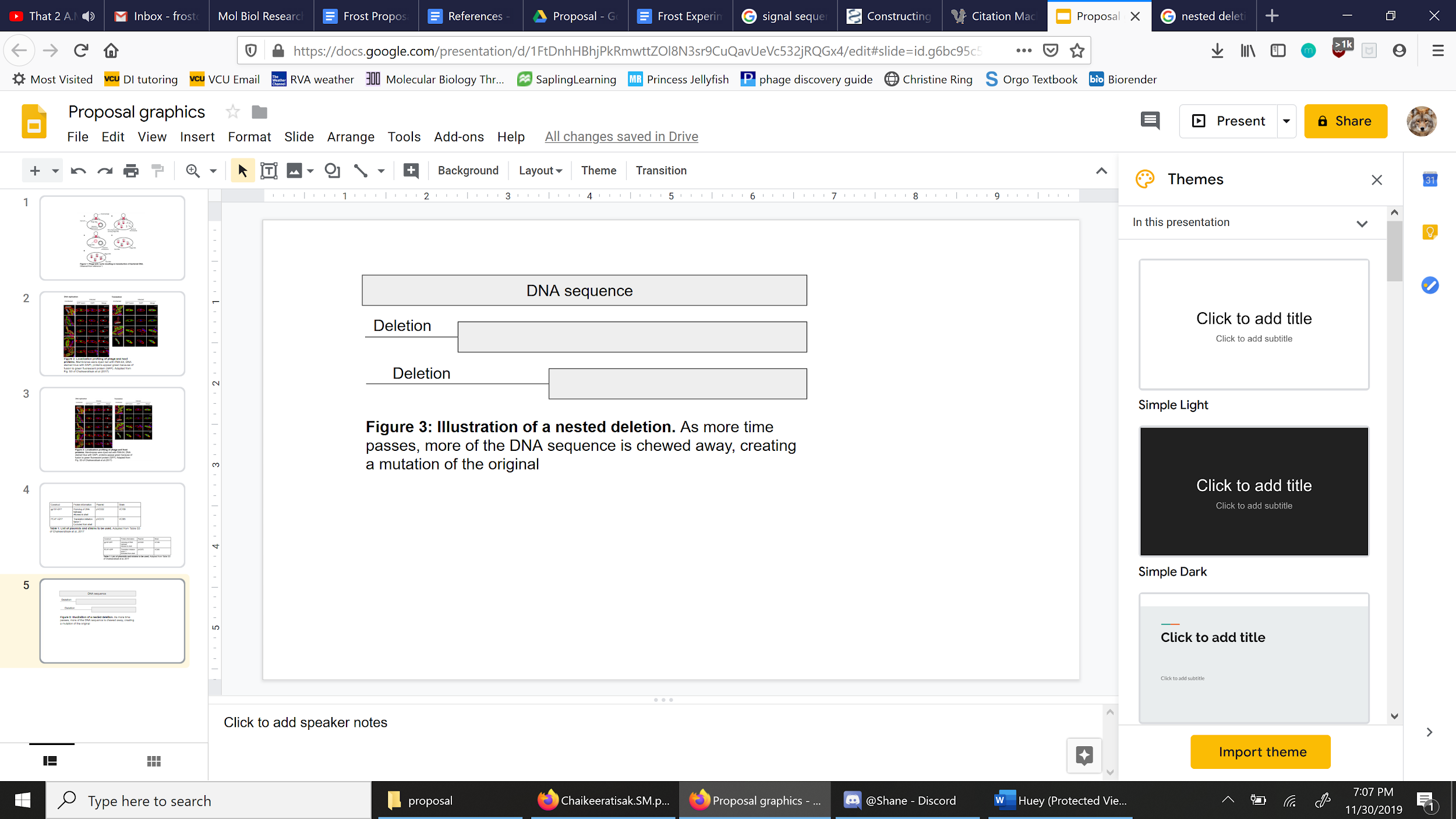
Later, GFP fusions to 52 phage proteins (that had previously been identified using mass spectrometry) were created and observed using localization profiling. From this experiment, it was observed that phage DNA and a handful of the phage proteins were localized within the gp105-GFP shell while others remained on the surface of the structure or floating in the bacteria’s cytoplasm (Chaikeeratisak et al, 2017). The phage proteins allowed into the shell tended to be those associated with DNA replication and transcription. While those congregated along the shell’s surface tended to be those associated with DNA translation. For example, gp197-GFP[[4]](#footnote-3) was allowed into the shell while IF1-GFP[[5]](#footnote-4) was excluded (Chaikeeratisak et al, 2017).

From an evolutionary aspect, it is theorized that this structure may serve to protect phage DNA and proteins from the bacteria’s defense mechanisms. By understanding it and the mechanisms by which it functions it may one day be possible to destroy unhelpful/harmful bacteriophage by creating mutations in the proteins they express. During the initial experiments with the gp105-GFP shell, the researchers decided to mutate cytoskeletal protein fusion mCherry-PhuZ to remove the ability to hydrolyze guanosine triphosphate (GTP). The goal in doing this was to test the role of mCherry-PhuZ in the process of the formation and movement of the gp105-GFP shell. The resulting mutated protein was unable to perform dynamic movement and the gp105-GFP shell became stuck at one of the cell’s poles (Chaikeeratisak et al, 2017). With more experimentation and information about the proteins and mechanisms involved in the lytic cycle of *P. chlororaphis* phage 201phi2-1 it could be possible to create mutations that would render lytic growth impossible--ultimately rendering the phage useless. *Pseudomonas chlororaphis* is used to prevent certain fungal diseases in an agricultural setting (Chin-A-Woeng et al, 2000). Halting the progress of phage working to destroy these populations could thus be beneficial.

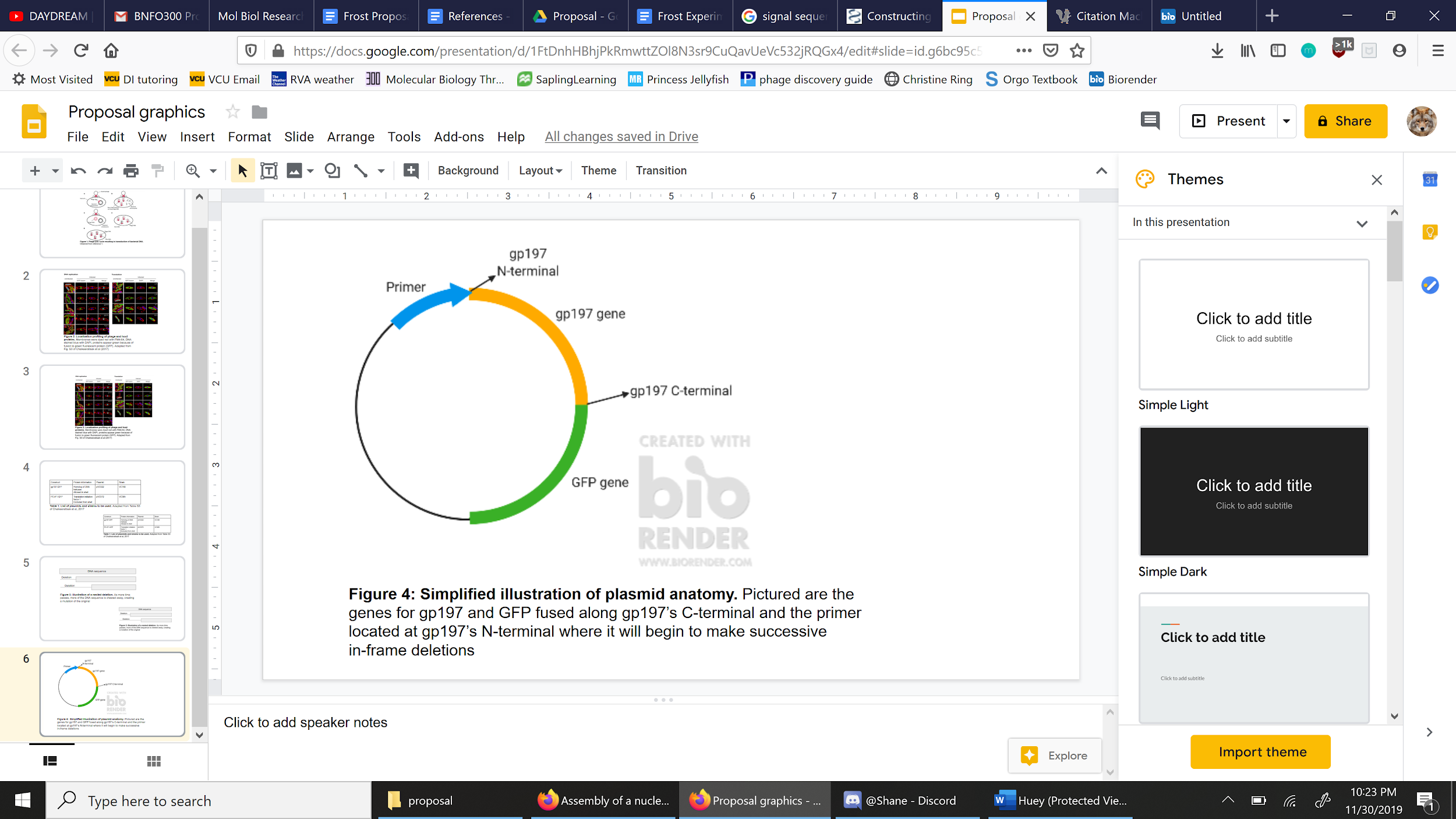
Alternatively, employing an improved understanding of the proteins and mechanisms used by phage201phi2-1 could be used to fight against bacterial infections. Phage therapy[[6]](#footnote-5) is still a new and experimental field, but it shows promise. A handful of patients in the throws of multi-drug resistant infections have been helped by the administration of phage cocktails (Furfaro et al, 2018).

**II. Experiment**

The proposed experiment will endeavor to explain why certain proteins are allowed within the proteinaceous shell while others are excluded by attempting to identify a shared amino acid sequence among the proteins permitted to enter the structure. While there is the possibility that entry is given randomly, the evidence of organization inside the shell based on function suggests the existence of a mechanism involved in the sorting process (Chaikeeratisak et al, 2017). 

In order to determine the function of most anything in molecular biology, the simplest thing to do is to mutate (break) it and see what happens differently. To test for the presence of an amino acid “entry” sequence, the genomic sequence of one or more phage 201phi2-1 proteins will need to be mutated. A mutated genome will not be able to code for the same amino acid sequence as its wild type. 

For this experiment, plasmids will contain a selected phage protein’s gene fused to the gene for GFP on the protein gene’s C-terminus (Table 1). These plasmids will have primers for nested deletion[[7]](#footnote-6) added on the protein gene’s N-terminus using an inverse polymerase chain reaction (PCR)[[8]](#footnote-7). This process, repeated several times, will produce multiple partially deleted plasmids. Template DNA will be removed using DpnI: a restriction enzyme used to cleave methylated DNA (Patrick, 2016).

When all of the above is completed, the plasmids will be transformed into *Escherichia coli* cells where a handful will be checked to ensure that the expected deletions have occurred. More or less this step is here to ensure that from the beginning of the process to now nothing unexpected or problematic has occurred. It is necessary to know what is being put into the cells or tracking the cause of an effect will become next to impossible. If all appears well, the plasmids will be transformed into *Pseudomonas chlororaphis* cells. From there phage 201phi2-1 will be introduced so that the mutations can be observed during infection. The protein localization will be monitored using fluorescence microscopy and the results will then be compared against the results from Chaikeeratisak et al (2017).

**III. Discussion**

If there is an amino acid sequence determining a protein’s access permissions to the proteinaceous shell then at some point in the nested deletions the protein should lose its ability to enter the structure as the genetic information coding since the sequence will have been removed. However a protein losing its ability to access the shell structure is not a guarantee that an amino acid sequence directly determines whether or not it can enter the shell. It may be that only proteins of a certain shape, or containing an area with a certain shape, are allowed entry and that the loss of amino acids from the deletions changed the resulting protein enough that it could no longer form the correct shape.

If such results were the case then future experiments would likely expand on this methodology and run trials using all of the known proteins from phage 201phi2-1 allowed into the shell to see if each lost accessibility to the shell at a certain deletion point. If the results continued to be consistent comparing the amino acid sequences of these phage proteins using a computer program to look for matching sequences across all or most of the proteins would be a reasonable next step. Were a particular sequence to appear in a majority of the proteins, then the next steps could include running similar tests and comparative analyses on phage protein known to not be able to enter the shell.

If no difference in phage protein accessibility and localization is observed then it could indicate a few things. It may be that there was an error in the methodology. When something is changed, you expect its interactions with its environment to change. If a dog loses a leg it certainly isn’t going to leap about like it did when it had all four. A mutated protein acting just as a wild type does suggests that perhaps it is not mutated after all. Another possibility is that there isn’t a mechanism by which phage proteins are sorted in the shell and its by chance that the ones that do enter the shell make it. This option seems highly unlikely considering the results of Chaikeeratisak et al (2017) which showed consistent organization of the same proteins. However, it remains an option, no matter how unlikely. Finally, such a result could indicate that while there is a mechanism involved in sorting phage proteins within the cell and the shell it is not related to the phage proteins themselves. Perhaps the host’s proteins and internal machinery are responsible for the sorting. Or maybe a different agent altogether is the deciding factor.

Were these results to be consistently obtained, and human error was controlled for, the next steps would be to check the methodology being used for possible errors in logic and/or to explore other possible mechanisms that could be sorting the phage proteins. One might compare protein localization between cells with wild type mCherry-PhuZ and the mutated version mentioned earlier to explore if cytoskeletal proteins play a role. Mutations could be introduced to gp105-GFP to see how changing the shell’s structure affects which proteins enter it and which don’t.

Ultimately, no result is a bad one. The goal of science is to uncover the truth and build an accurate understanding of the world around us. Whatever the outcome of the experiment, more information will have become available for others to ponder and use to develop experiments of their own.

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1. Transduction, phage-facilitated transfer of genes from one bacterium to another, is one of the mechanisms by which pathogenicity can spread from one bacterial strain to another (Kasman & Porter, 2019) [↑](#footnote-ref-0)
2. Refers to the lysogenic phage life cycle wherein a phage, instead of undergoing the lytic cycle, integrates its own genome into the bacterial genome (Kasman & Porter, 2019) [↑](#footnote-ref-1)
3. Level of expression determined using mass spectrometry, see reference 12 for more information on the principles and methods of mass spec. [↑](#footnote-ref-2)
4. gp197 is a homolog of DNA helicase (Chaikeeratisak et al, 2017) [↑](#footnote-ref-3)
5. IF1 = translation initiation factor 1 [↑](#footnote-ref-4)
6. Using cocktails of bacteriophage to treat bacterial infections, usually those that have become resistant to antibiotics (Kasman & Porter, 2019) [↑](#footnote-ref-5)
7. A set of deletions allowed to run from the same point for variable amounts of time, creating plasmids holding varying amounts of the same DNA sequence, for more information see figure 3 and reference 13 [↑](#footnote-ref-6)
8. For more information on inverse PCR see reference 10 [↑](#footnote-ref-7)