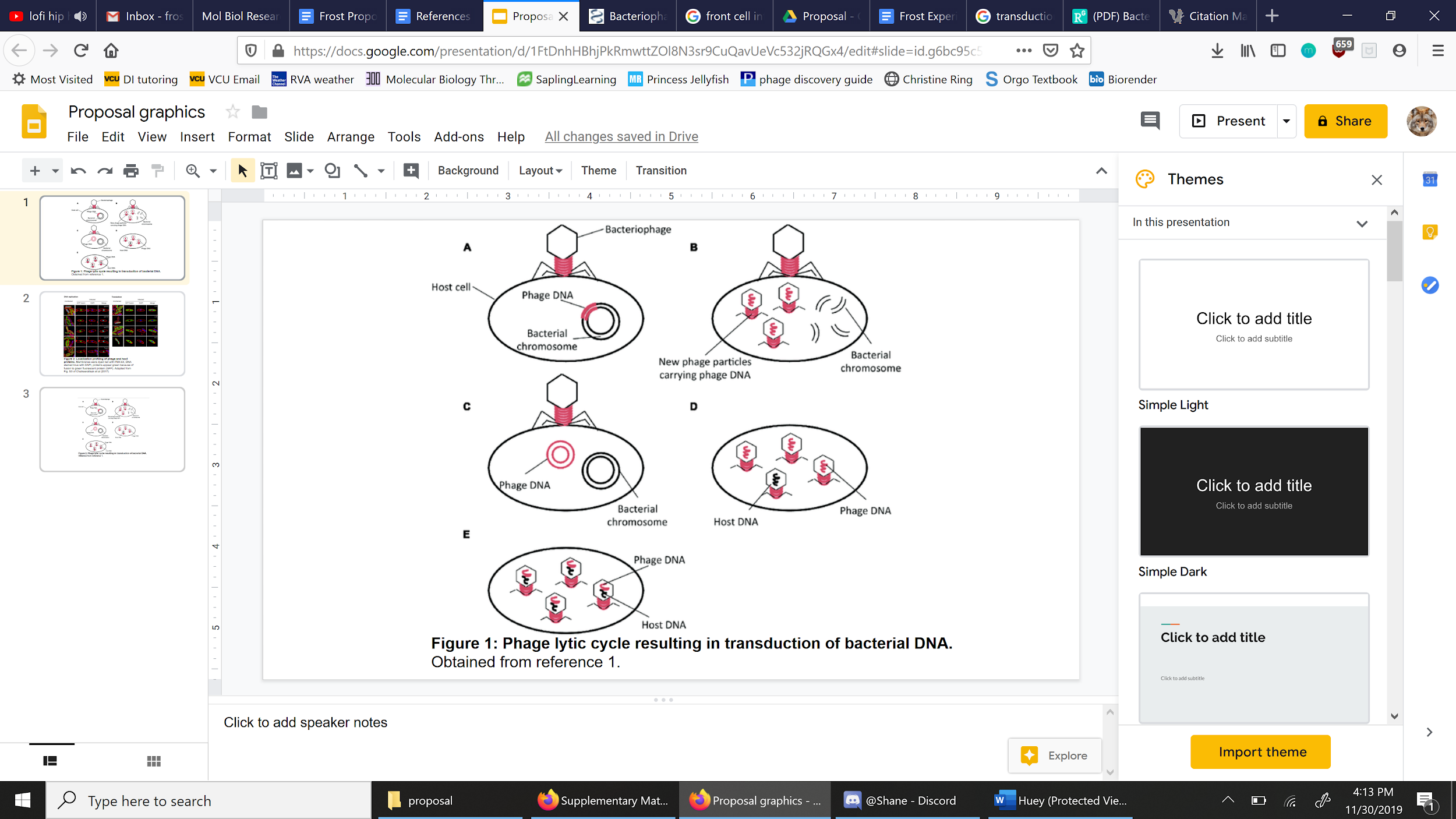
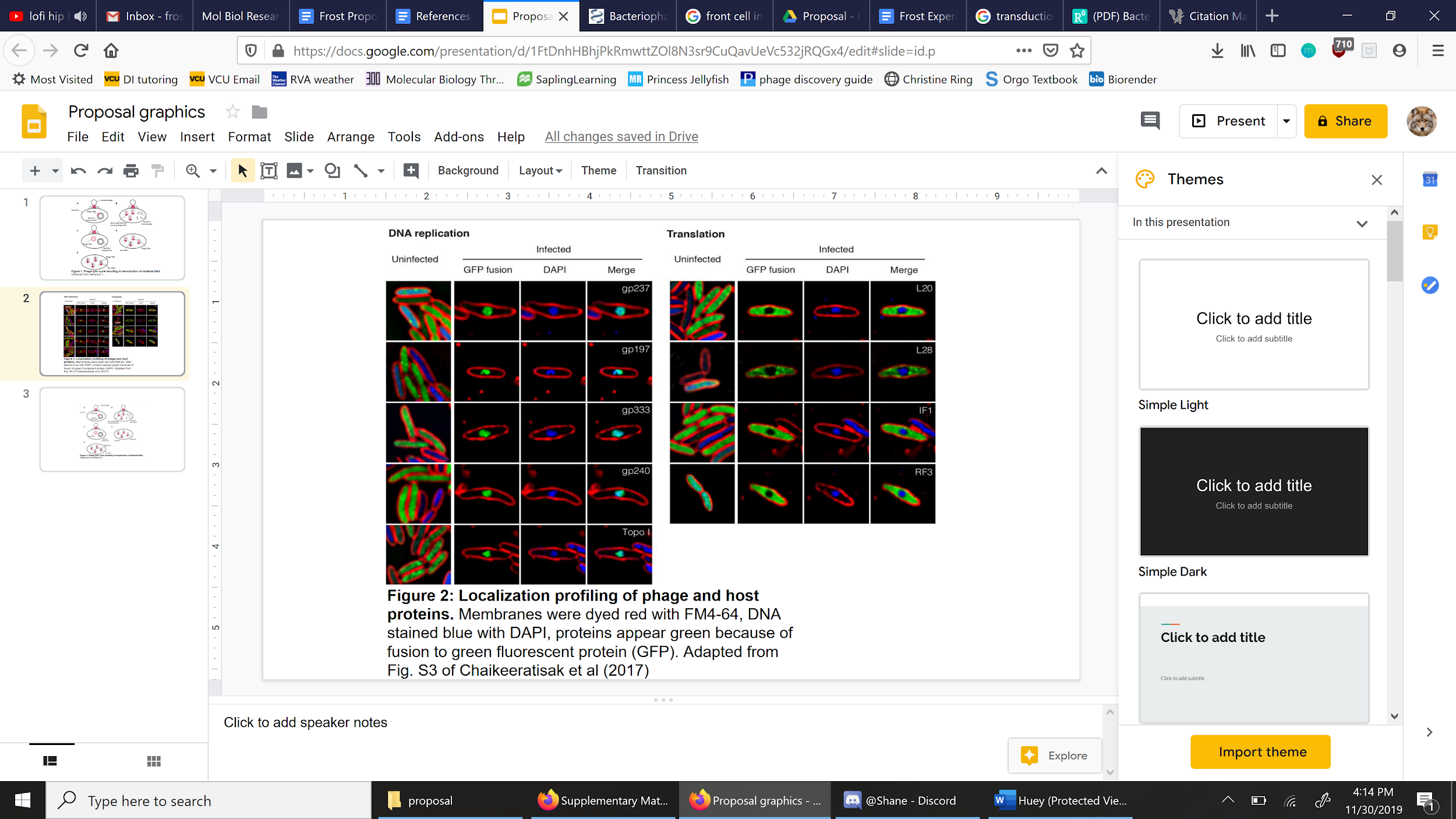
The Mechanism by Which Pseudomonas Chlororaphis Phage 201Φ2-1 Proteins are Sorted within the Phage-Formed Proteinaceous Shell

**I. Introduction**

Bacteriophages (“phages”) are viruses that have evolved to exclusively target bacterial cells. Each phage species is highly specialized and attacks only a narrow range of bacterial species. Some are so selective they will only use specific strains of bacteria as hosts. 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. 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 [8]. 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.[[1]](#footnote-0)

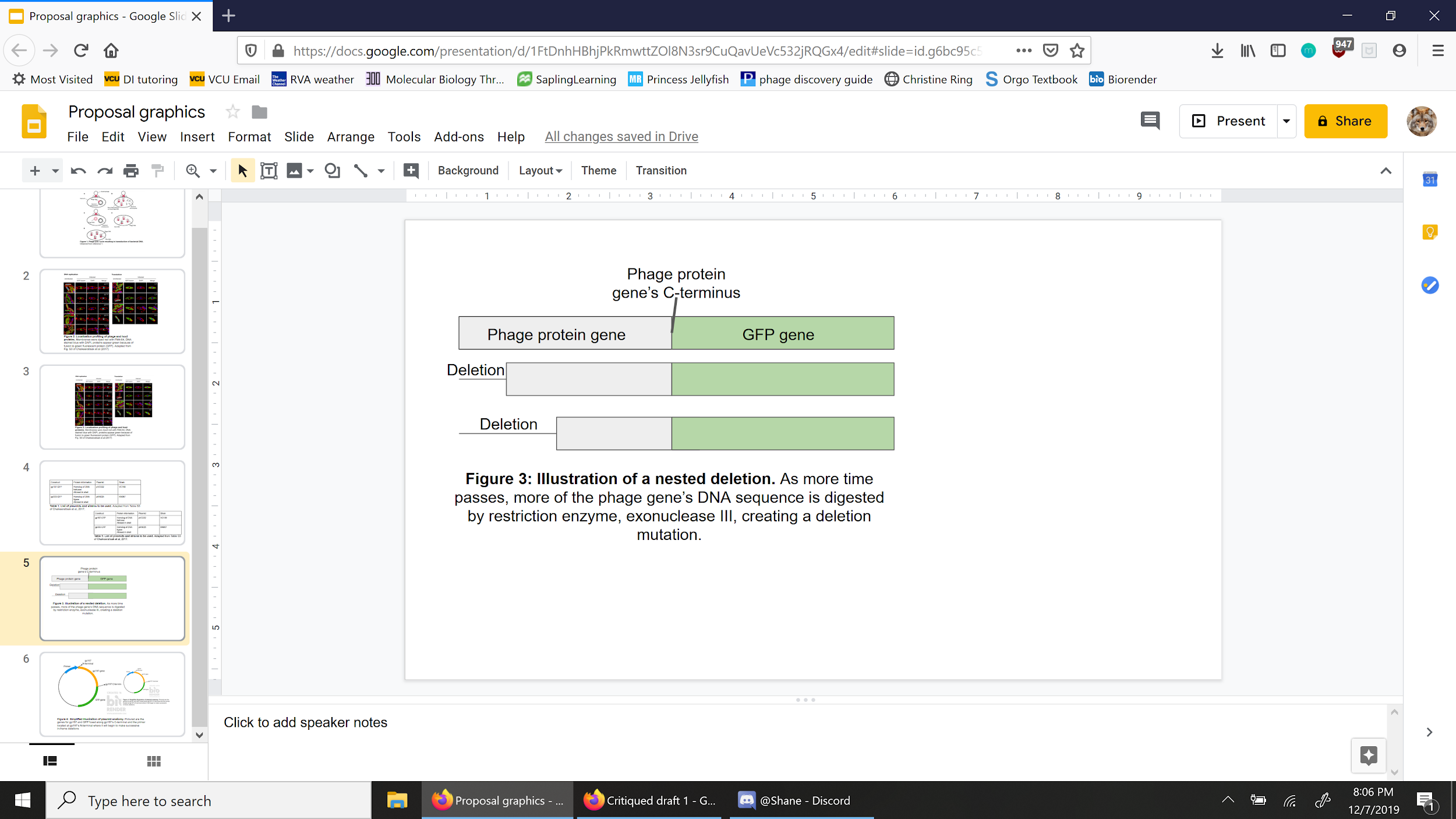
Phage generally reproduce using the lytic cycle (**figure 1**) wherein they hijack their host’s reproductive machinery to create new copies of themselves in the cytoplasm of the host [9]. They do this by first attaching to the bacterial cell’s membrane and injecting their DNA into the cell’s cytoplasm. From there, the bacteria’s resources are diverted to phage production until the cell lyses (“bursts”) and dies [9]. Recently, however, *Pseudomonas chlororaphis* (*P. chlororaphis*) phage 201ϕ2-1 (alternately written as 201𝜑2-1 or 201phi2-1) was identified as forming a protein shell within its host that was centered by cytoskeletal proteins [5]. Within the shell phage DNA, certain phage proteins, and a handful of host cell proteins were identified. Such a mechanism had never been observed and has yet to be explained.

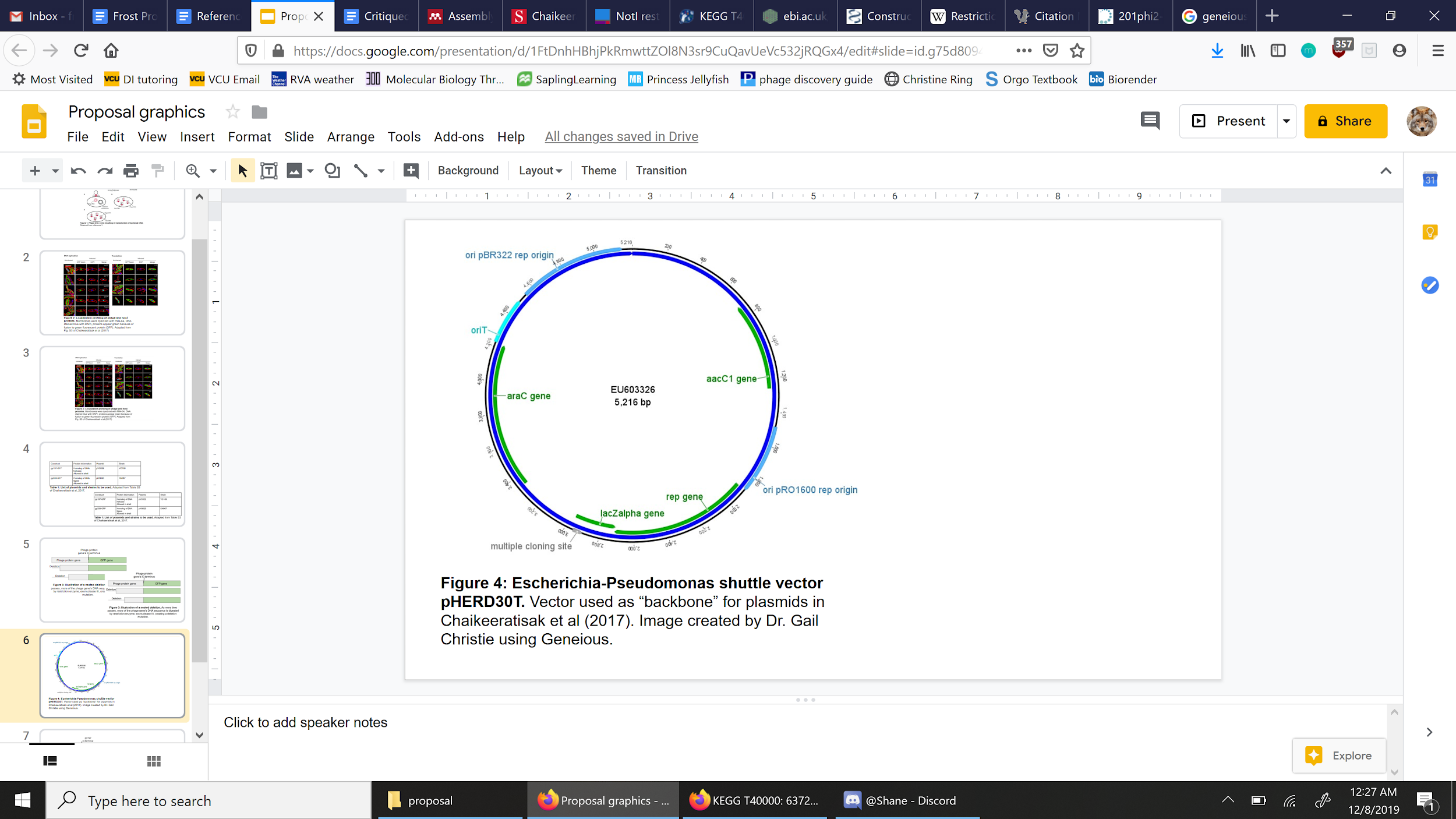
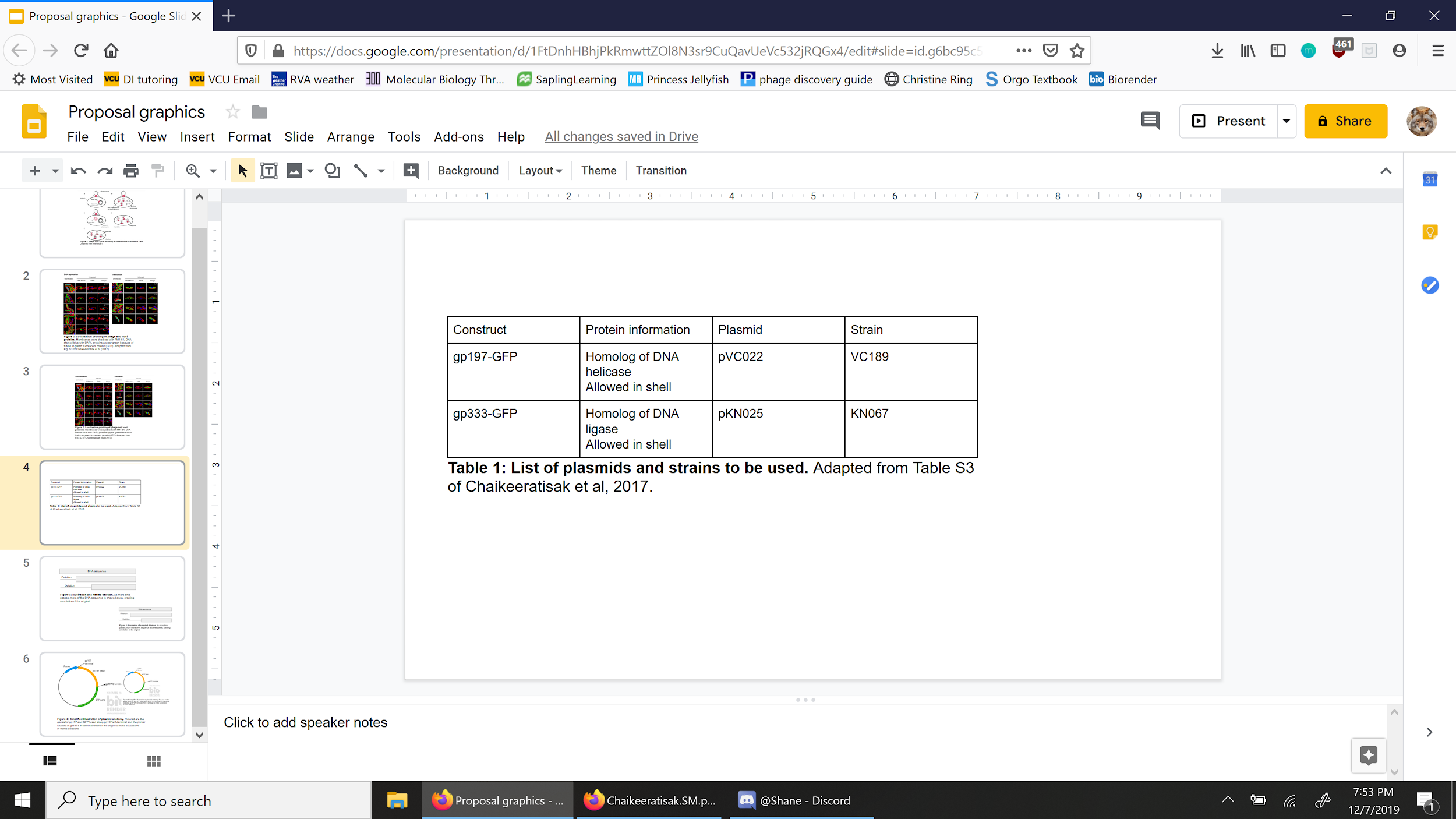
Chaikeeratisak et al (2017) discovered this nucleus-like structure by creating fusions between green fluorescent protein (GFP) and gp105 (a highly expressed phage protein[[2]](#footnote-1)) and observing the fusions’ behavior within the cell using localization profiling. As infection progressed, gp105-GFP was shown to form a small focal point at one of the cell’s poles before being moved to the midcell by cytoskeletal protein fusion mCherry-PhuZ[[3]](#footnote-2) [5]. At the midcell the mass continued to grow [5]. 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 [5].

After discovering the phage-formed protein shell, Chaikeeratisak et al (2017) created a fusion between phage DNA and DAPI (a blue fluorescent protein), GFP fusions to some host proteins, and GFP fusions to 52 phage proteins (that had previously been identified using mass spectrometry) and observed them using localization profiling (**figure 2**). In doing so, the goal was to identify other elements that interacted with the protein shell. From this experiment, it was observed that phage DNA, a handful of host proteins, and some 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 [5]. The 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 [5].

Halting the progress of phages working to destroy *Pseudomonas chlororaphis* populations could be beneficial because *P. chlororaphis* is used to prevent certain fungal diseases in an agricultural setting, [5]. With more experimentation and information about the proteins and mechanisms involved in the lytic cycle of phage 201phi2-1, it could be possible to create mutations that would render lytic growth impossible--ultimately rendering the phage useless. In order to begin to better understand phage 201phi2-1, I would like to explore the possible mechanisms it employs to sort DNA and proteins into its shell. The proposed experiment will attempt to identify an amino acid sequence or sequences used by phage proteins to gain access into the shell structure.

**II. Experiment**

The evidence of organization inside the shell based on function suggests the existence of a mechanism involved in the sorting process [5]. Because of the role amino acids play in the shape and properties (hydrophobicity, charge, etc.) of proteins its reasonable to assume that might have a role in determining which phage proteins have access to a phage-formed proteinaceous shell. To test for the presence of an amino acid “entry” sequence, the genomic sequences of two phage 201phi2-1 proteins--gp197 and gp333--known to access the protein shell will be mutated using nested deletions (**figure 3**). The deletions should remove genetic code for amino acid sequences. To prevent the reading frame from being shifted, nucleotides will only be deleted in multiples of 3. Because the mutated genomes will not be able to code for the same amino acid sequence as their wild types, there should be an observable difference between the localization of the mutated phage proteins and their wild type counterparts.

Mutations will be created using site-directed mutagenesis through inverse PCR. Plasmids designed in Chaikeeratisak et al (2017)[[6]](#footnote-5) for gp197 and gp333 (**figure 4**, **table 1**) will be used in tandem with primers designed for this experiment. The primer pointed in the 3’ direction (away from the genes of interest) will contain the ribosome binding site and the phage protein gene’s start codon (ATG). A second primer, facing the 5’ direction (towards the genes of interest), will be designed with a 15 nucleotide overlap (for gp197: ATG GCT TCT CCC AAA) (for gp333: ATG TCA AAT CGC CAT) to the first primer. Exonuclease III will be the enzyme used to create the deletion mutations. Five separate deletions of 30 nucleotides each will be created using these materials combined with inverse PCR. Template DNA leftover from the PCR reaction will be cleaved using DpnI (a type IIM restriction endonuclease that recognizes and cleaves methylated DNA), which will then be inactivated with a heat shock [12]. Target DNA fragments will be recircularized using Gibson Assembly kits[[7]](#footnote-6).

When all of the above is completed, the plasmids will be chemically transformed into competent *Escherichia coli* cells (provided with Gibson Assembly kit). The E. coli cells will then be plated onto antibiotic aacC1. A handful of mature cells from the plate 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 competent *Pseudomonas chlororaphis* cells[[8]](#footnote-7). 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. A mutated protein acting just as a wild type does suggests that perhaps it is not mutated after all. It may be that deletions did not affect enough of the proteins’ genes. 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 that interact with the shell. 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. Refers to the lysogenic phage life cycle wherein a phage integrates its own genome into the bacterial genome [8] [↑](#footnote-ref-0)
2. Level of expression determined using mass spectrometry, see reference 12 for more information on the principles and methods of mass spec. [↑](#footnote-ref-1)
3. mCherry is a red fluorescent protein, PhuZ is a cytoskeletal phage protein [5] [↑](#footnote-ref-2)
4. gp197 is a homolog of DNA helicase [5] [↑](#footnote-ref-3)
5. IF1 = translation initiation factor 1 [↑](#footnote-ref-4)
6. To read specific methodology, see supplementary material for [5] [↑](#footnote-ref-5)
7. Kits include exonuclease, DNA polymerase, DNA ligase, and a buffer component [↑](#footnote-ref-6)
8. For more information on how to create competent cells see reference 2 [↑](#footnote-ref-7)