Viruses from Hell

My Summer with SSV1

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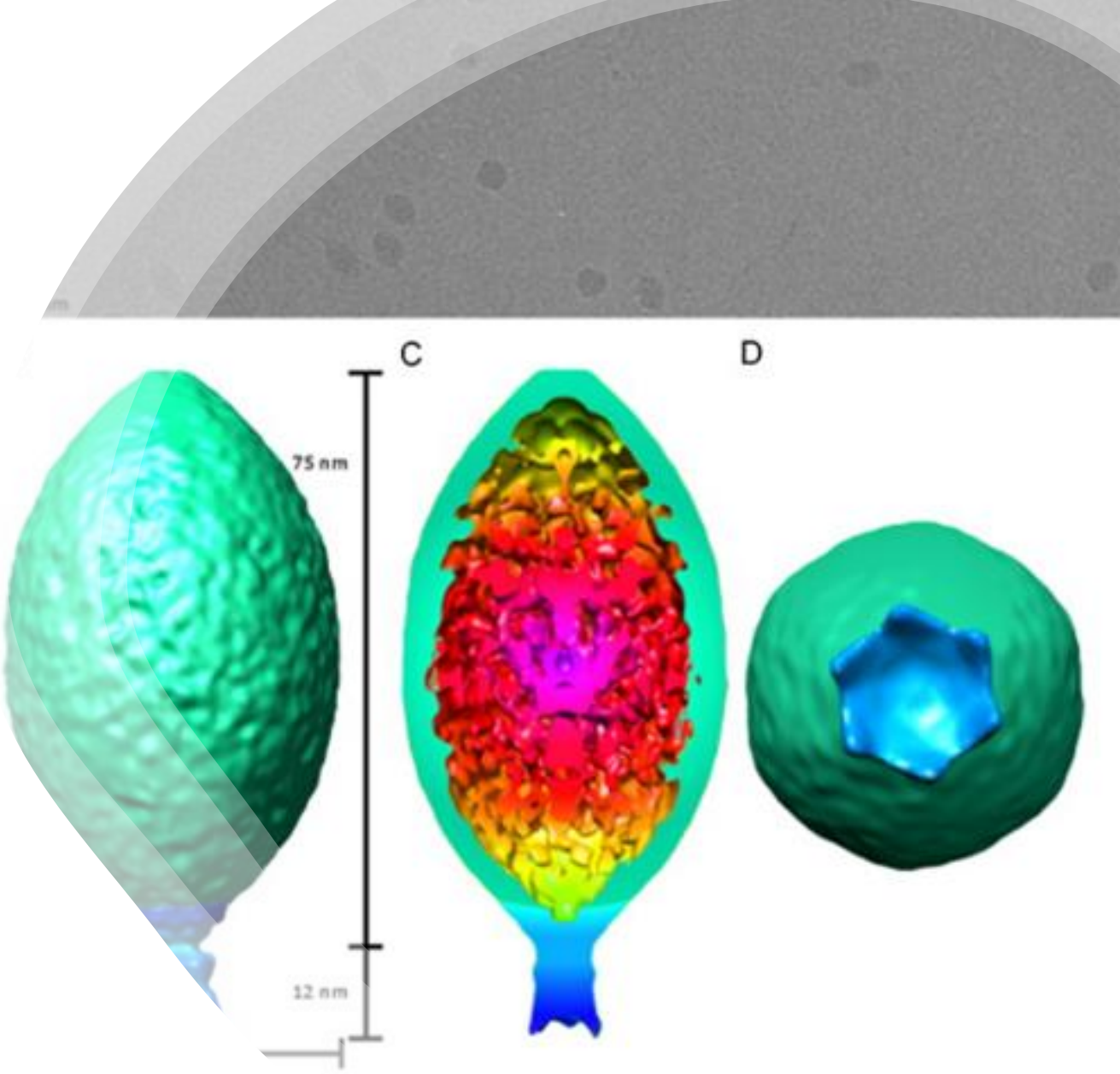
What are SSV1s?

Spindle Shaped Virus 1

- Extremophilic
- Lives in hot springs around the world
- Tolerance for extreme conditions attributed to capsid structure
  - Three major capsid proteins
- Natural host: archaea *Saccharolobus solfataricus*



SSV1 Structure from Stedman et al. 2015

The Objective:

To capture the elusive capsid protein structure of Spindle Shaped Virus 1

The Plan

Step 1

Grow SSV1s with cysteine mutations in VP 3 and VP 4

Step 2

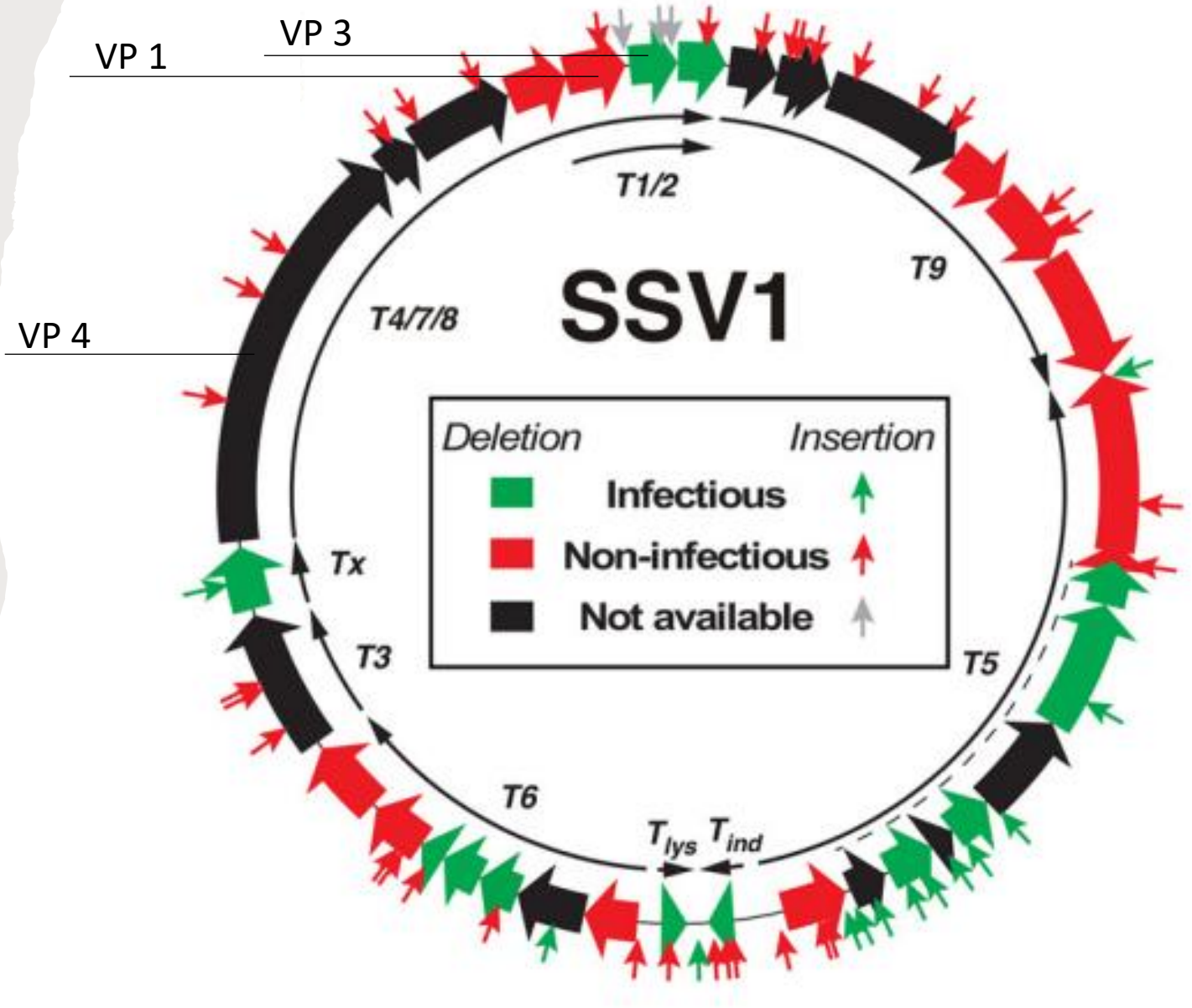
Prepare virus mutants with nanogold® which will stick to the cysteine

Step 3

Image the gold-labeled viruses with a TEM and isolate the positions of VP3 and VP 4

Focused on Four Strains of SSV1

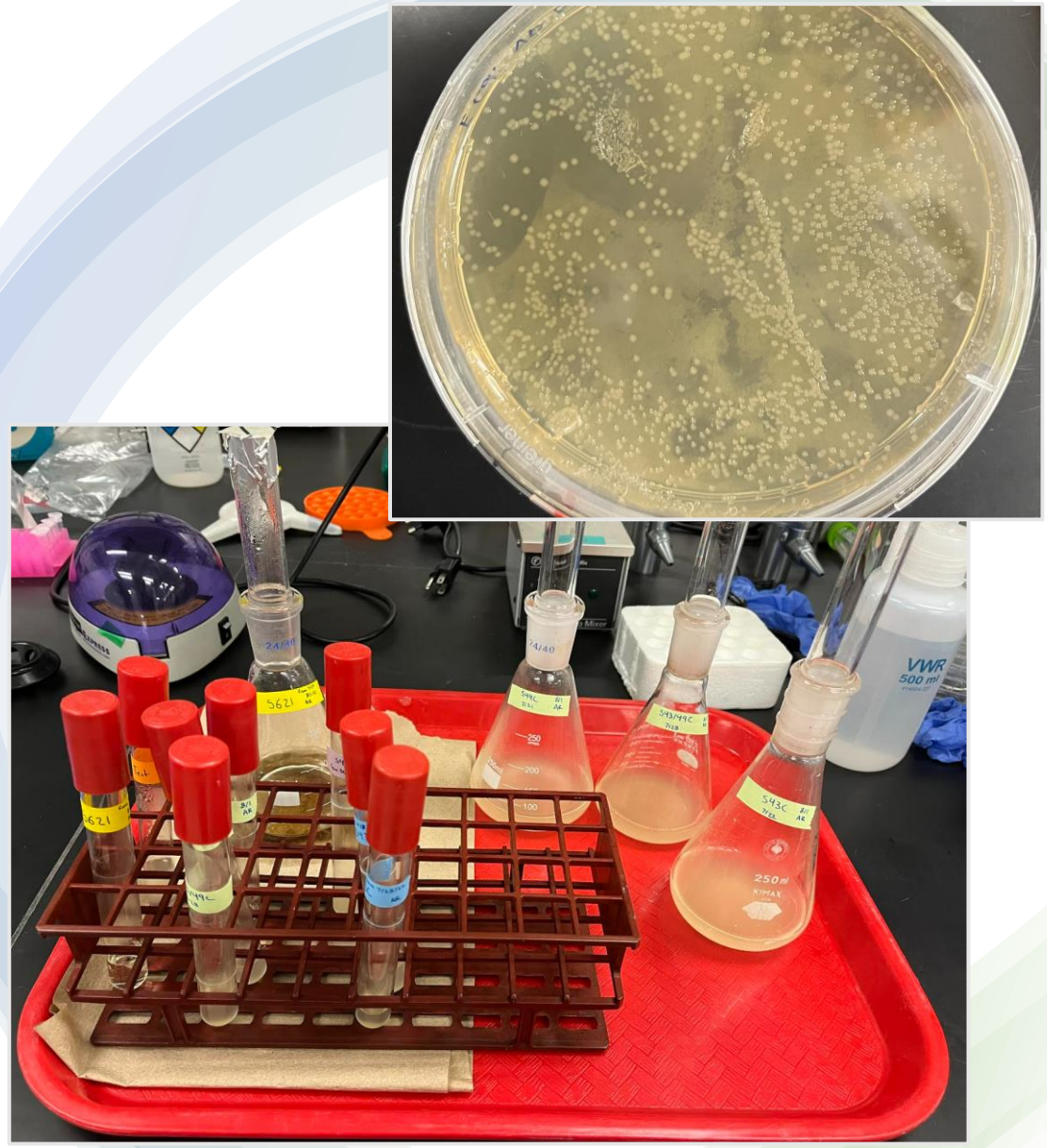
- Three Strains with Mutations in VP3
- One Strain with Mutation in VP4



Annotated SSV1 Genome from Iverson et al. 2017

Step 1:
Growing the SSV1

- Outgrew *E.coli* containing SSV1 mutant DNA
- Used electroporation to transfer virus DNA from the E.coli into *Saccharolobus* cells
- Grew *Saccharolobus* cultures infected with SSV1 strains

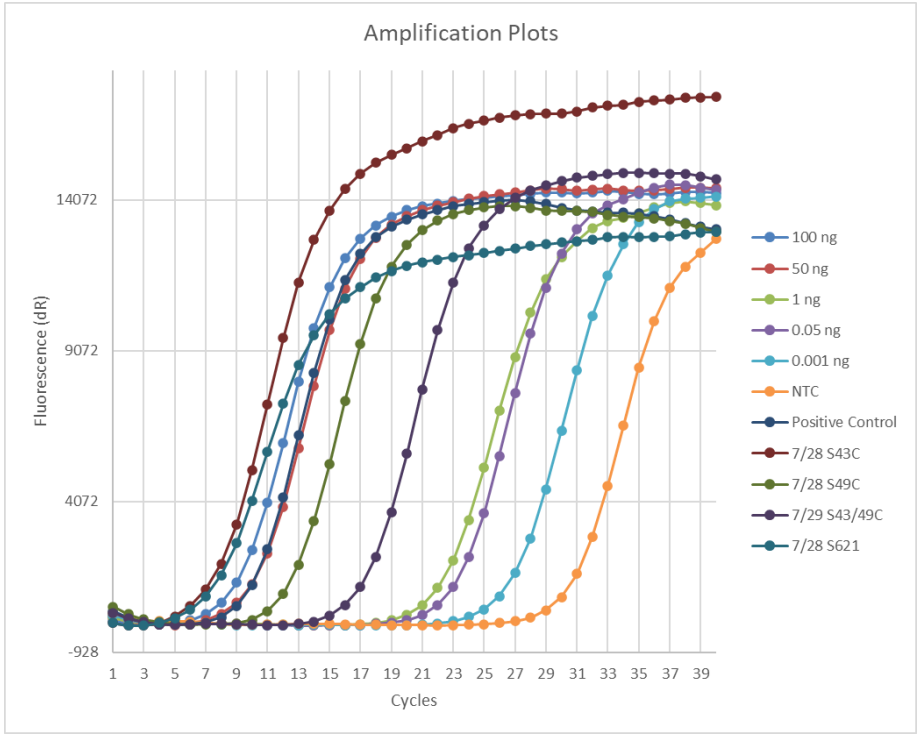


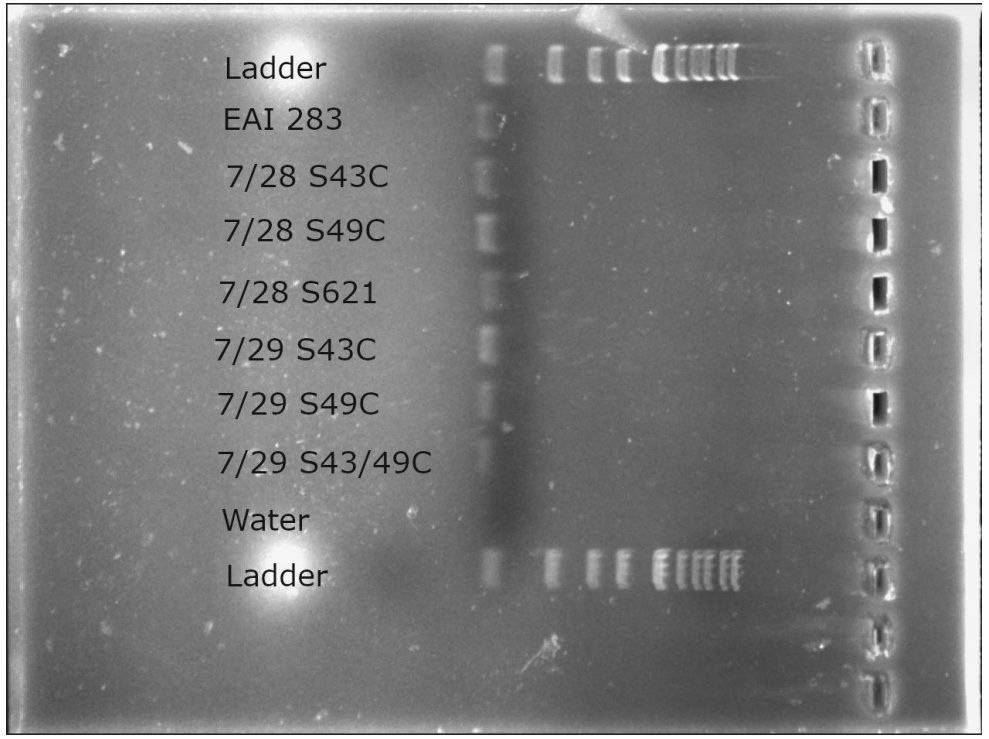
Checking Identity and Infectivity

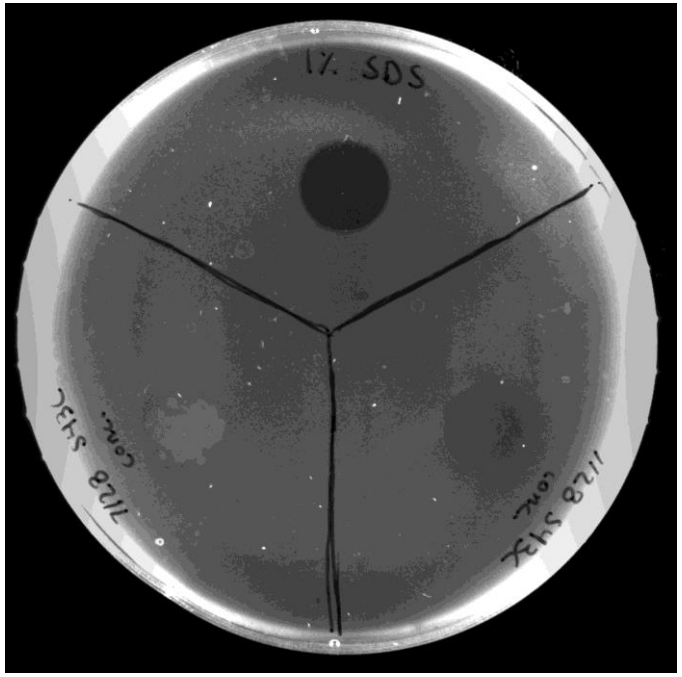
Techniques included polymerase chain reactions, halo assays, DNA sequencing, and TEM imaging of the virus

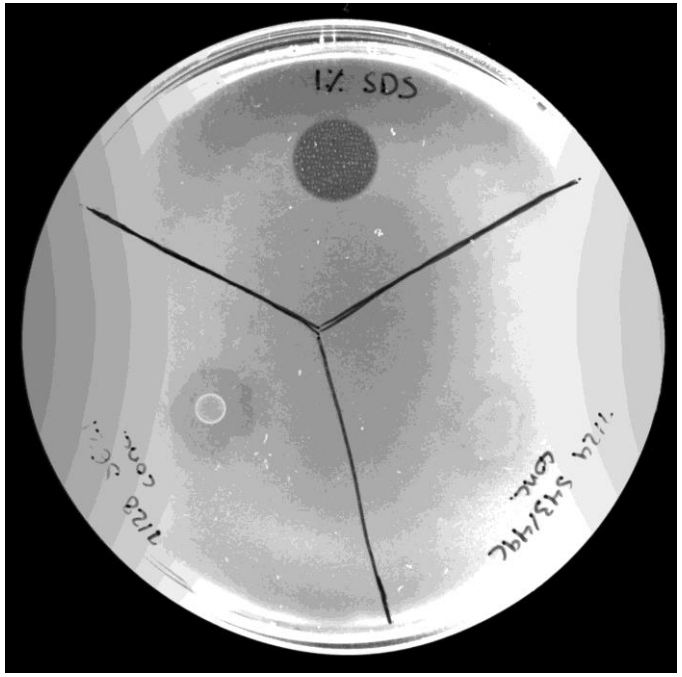
Polymerase Chain Reactions

- Used to confirm presence of virus
- Figure on left shows qPCR of concentrated mutants
  - qPCR additionally allows a quantitative analysis of the virus
- Figure on right shows PCR of concentrated mutants



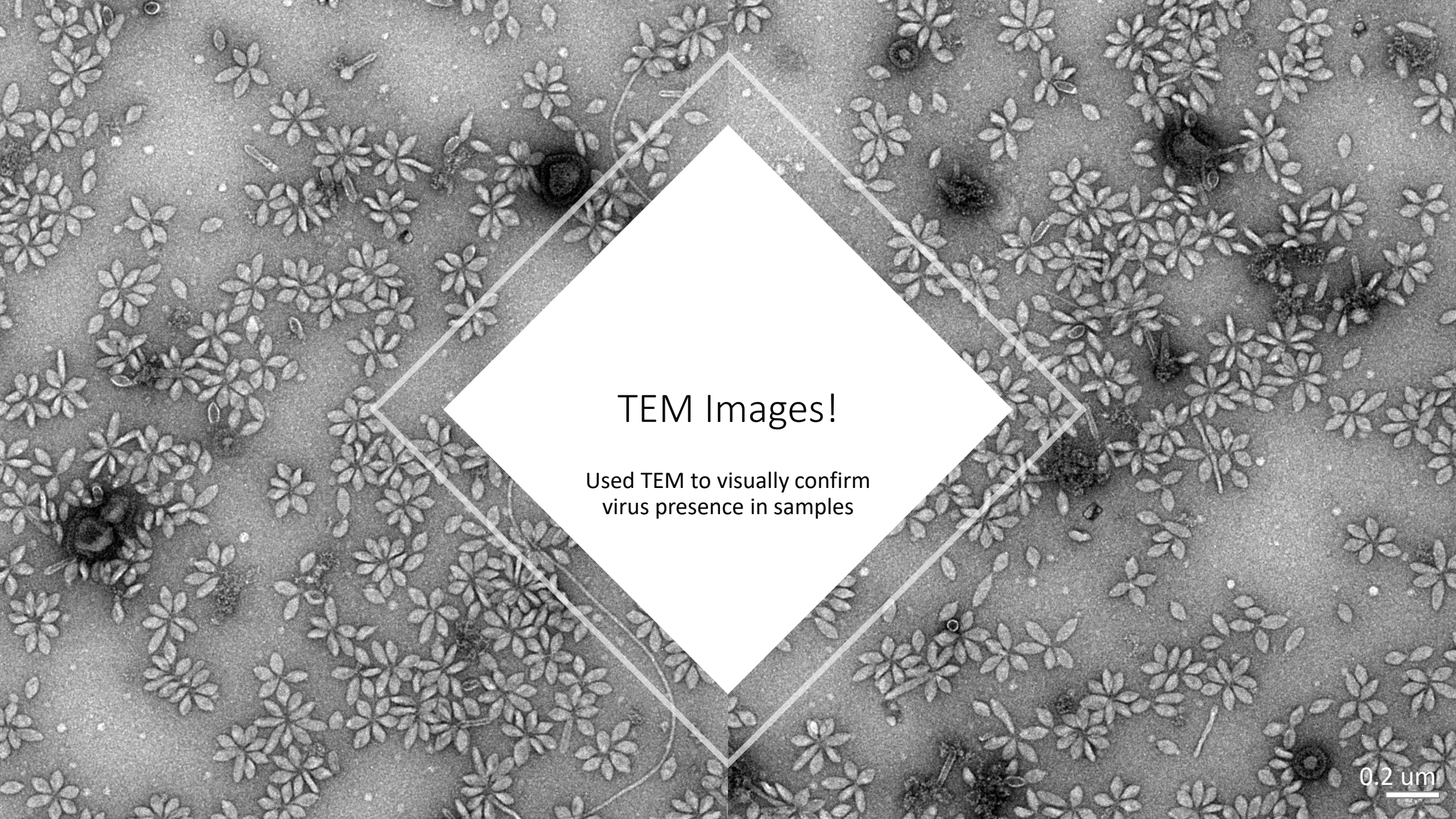






Halo Assays

- Used to confirm infectivity of the virus
- Top assay shows S43C and S49C with 1% SDS control
- Bottom assay shows S621 and S43/49C double mutant with 1% SDS control
- Faint halos can be seen for all concentrates
  - At this point all the mutant viruses had been grown and concentrated

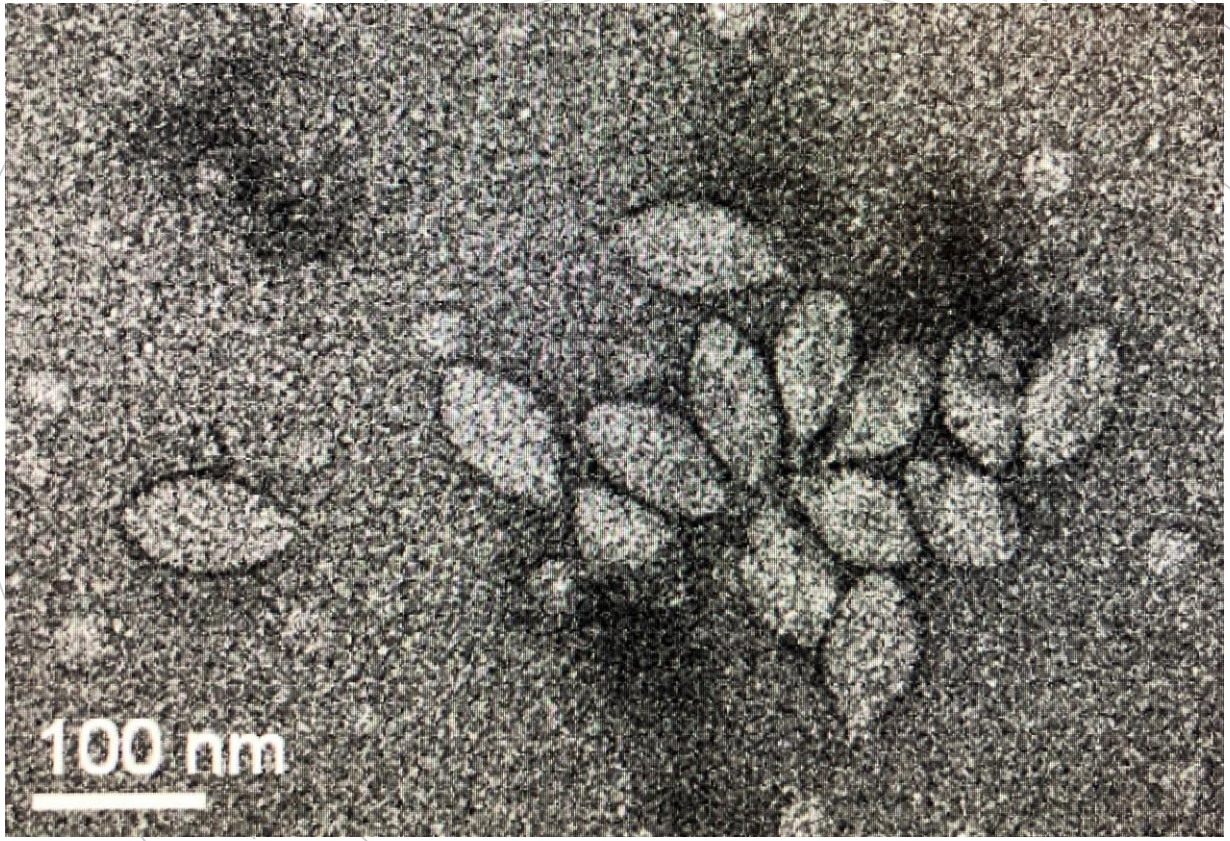


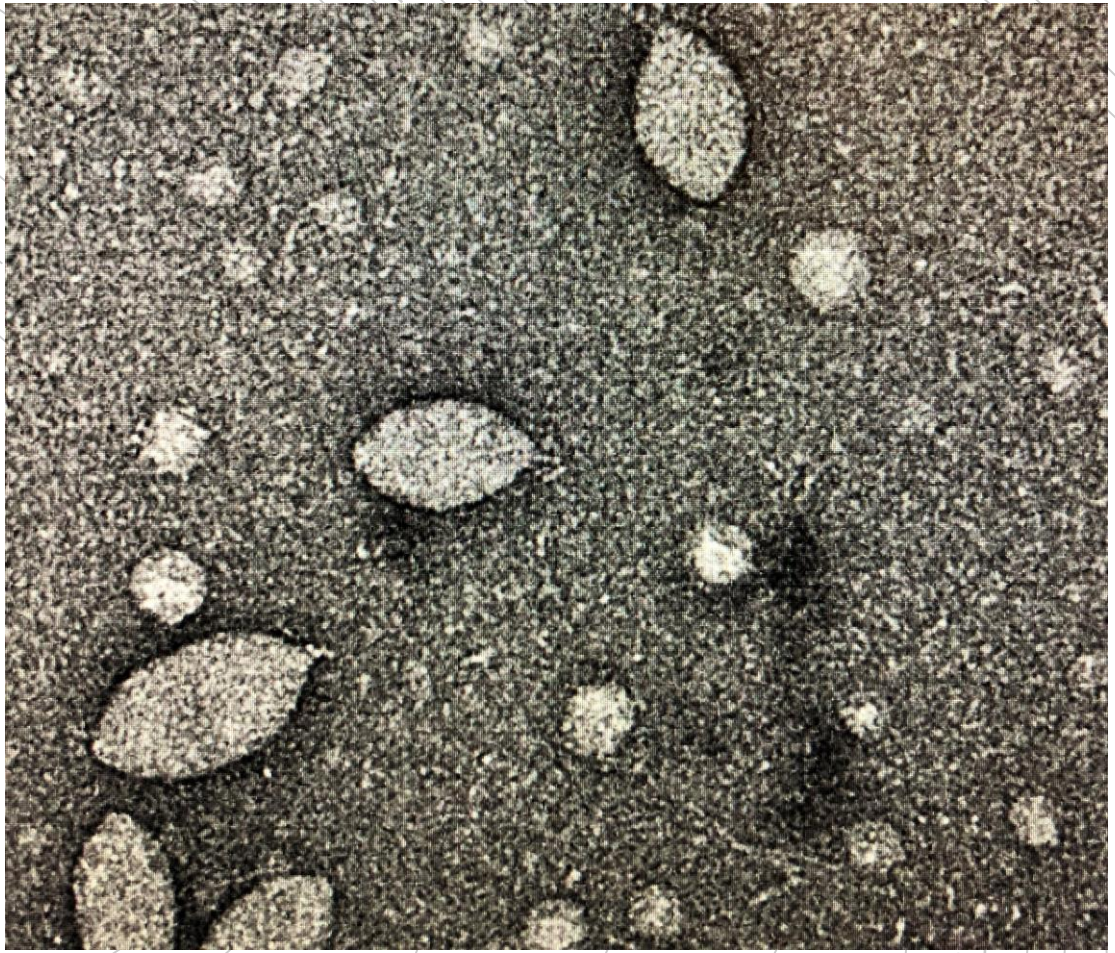
TEM Images!

Used TEM to visually confirm virus presence in samples

S49C As Seen in TEM

Final step in confirming the presence and abundance of SSV1 in a concentrate





Step 2:
Prep Virus Mutants with Nanogold® Particles

- The nanogold® particles bind to a thiol group in the cysteine
- Each nanogold® particle is 1.4nm in diameter
- In final solution, the number of nanogold® particles will be proportional 1:1 with the amount of cysteine

Step 3:
TEM Imaging of the Golden Proteins

- Like the nanogold® preparation, this step has yet to be completed
- Once stained the viruses will be examined with a TEM
- Each nanogold® particle is expected to show up as a black dot in the TEM
    - Position of the gold would be indicative of the virus protein's position in SSV1

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References

1. Stedman, Kenneth M., et al. “Structural Insights into the Architecture of the Hyperthermophilic Fusellovirus SSV1.” *Virology*, vol. 474, Jan. 2015, pp. 105–109., https://doi.org/10.1016/j.virol.2014.10.014.
2. Iverson, Eric A., et al. “Extreme Mutation Tolerance: Nearly Half of the Archaeal Fusellovirus Sulfolobus Spindle-Shaped Virus 1 Genes Are Not Required for Virus Function, Including the Minor Capsid Protein Gene *vp3*.” *Journal of Virology*, vol. 91, no. 10, 2017, https://doi.org/10.1128/jvi.02406-16.

The End

Any Questions?