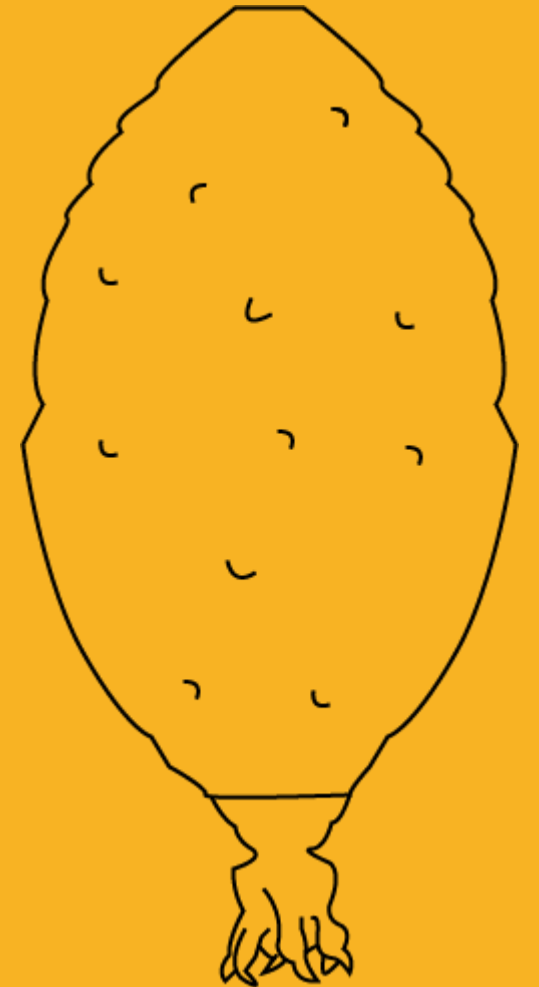


INTERACTION OF EXTREME VIRUSES WITH ARCHAEAL PROTEIN LAYERS



BY AUDREY HOULIS AND DR. KEN STEDMAN

Overview

Current Knowledge and Background

- *Sulfolobus* Spindle-Shaped Virus
- *Sulfolobus Solfataricus*

How Virus and Host Interact

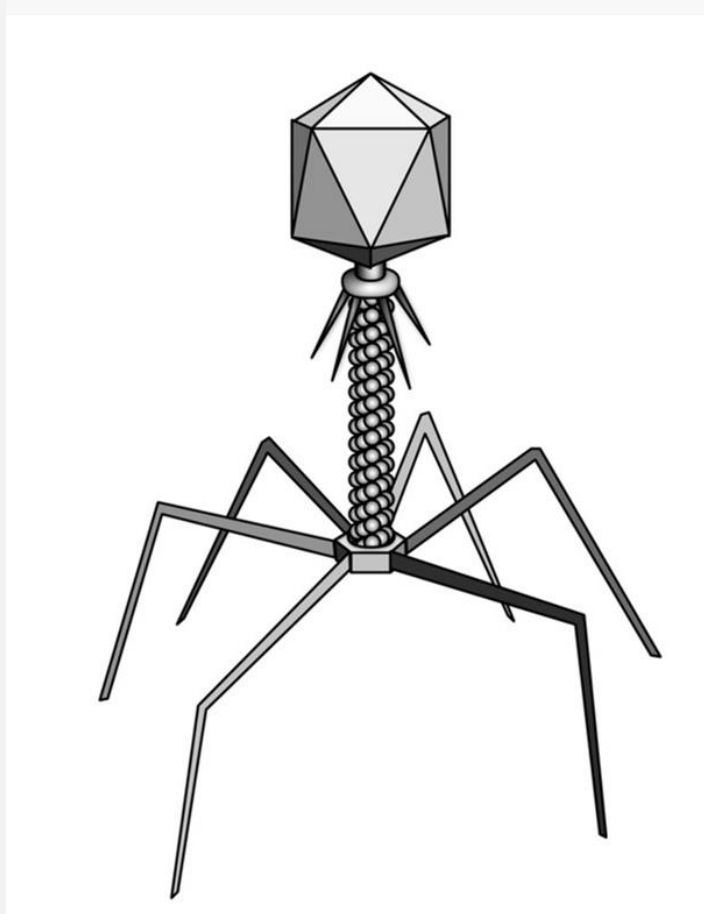
Experiment Methodology

Results and Next Steps

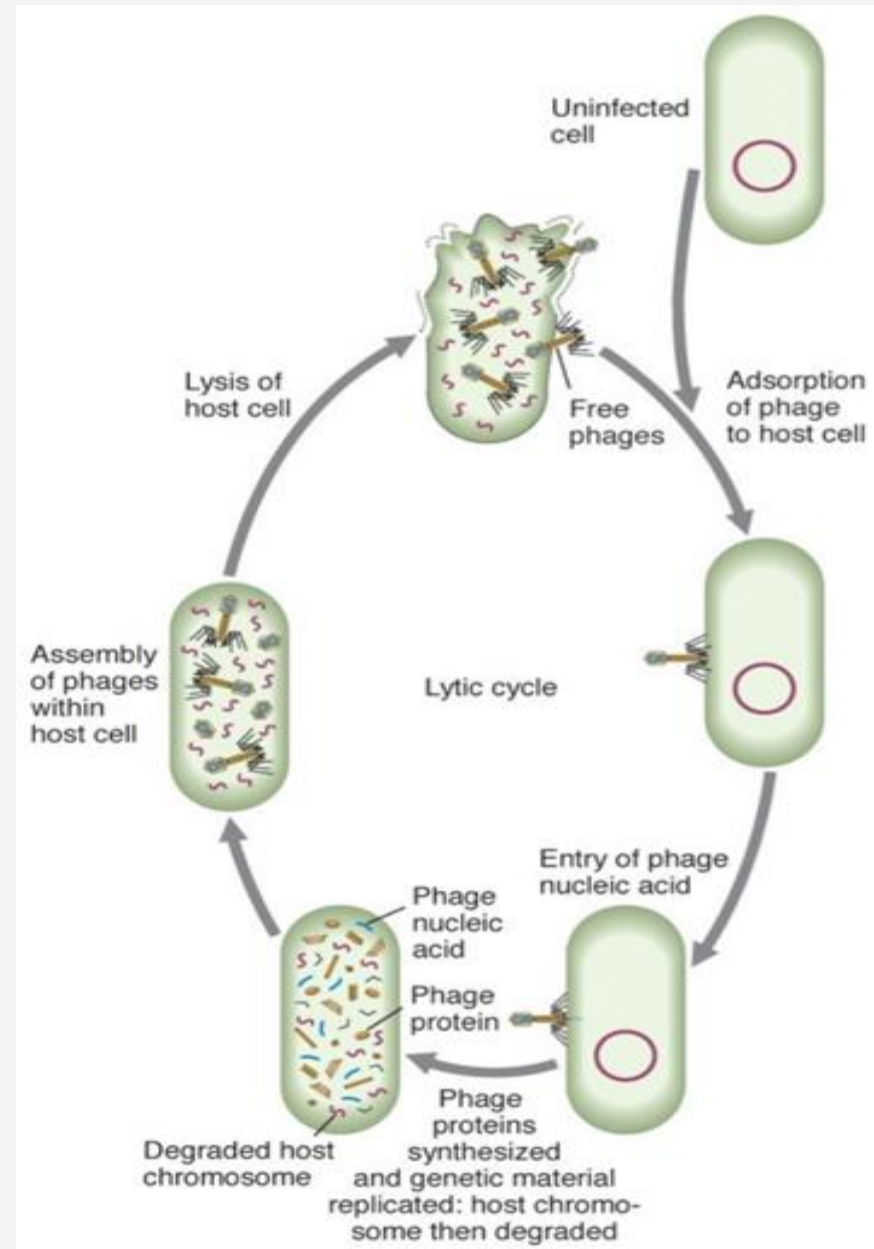


Lassen Volcanic National Park, Photo by National Park Service

What is a virus?

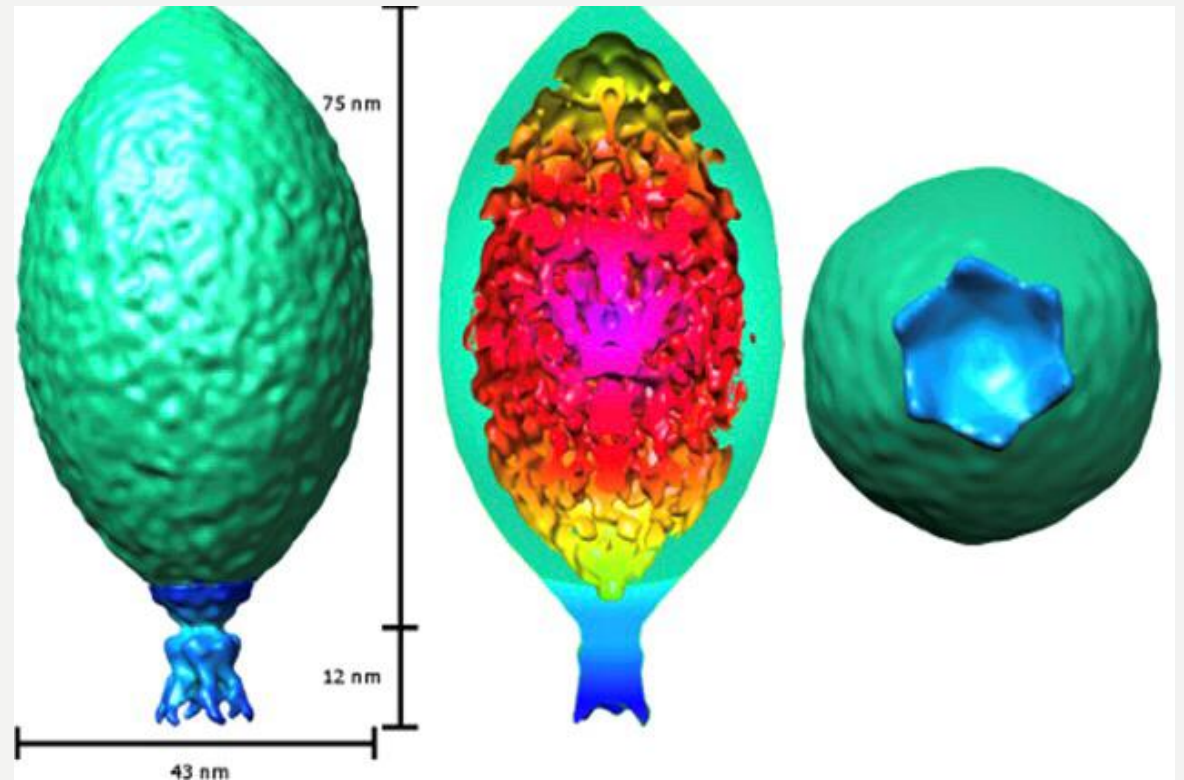


T4 Bacteriophage Virus



Background: *Sulfolobus* Spindle-Shaped Virus (SSV)

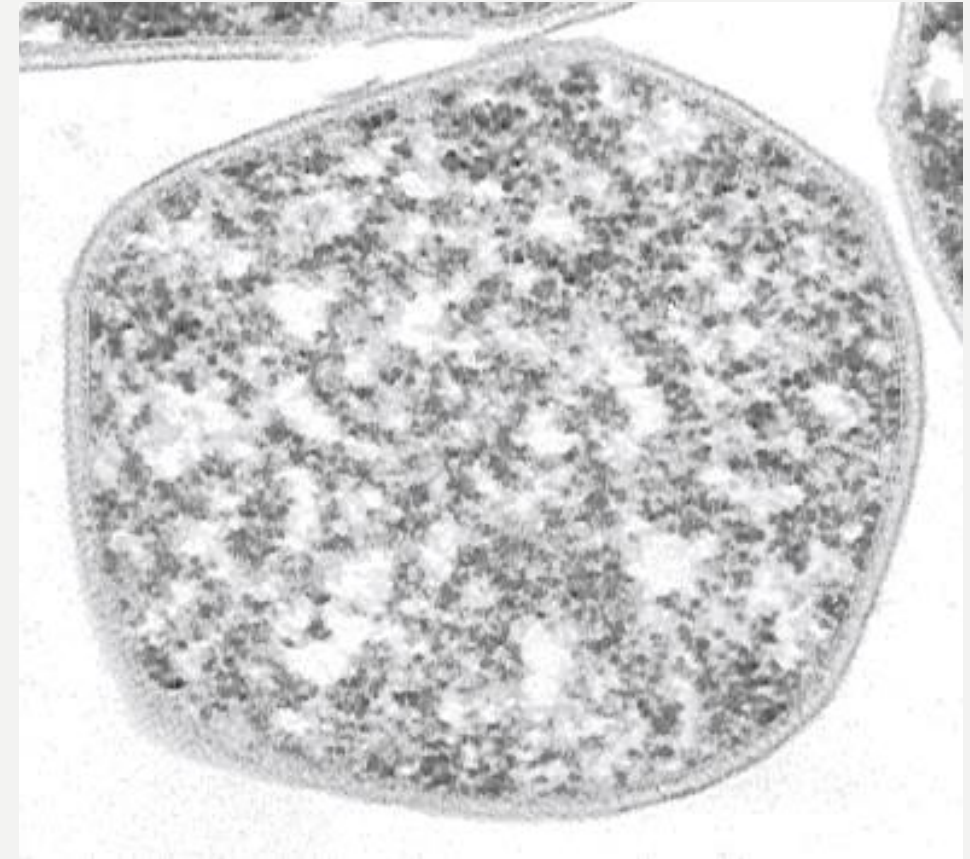
- SSV I extreme conditions:
pH 3, 80°C
- Archaea-specific
- Diverse shape
- Spindle-Shape
 - Unique tail structure
composed of 6 tail fibers



SSVI Structure from Stedman et al. 2015

Background: *Sulfolobus solfataricus*

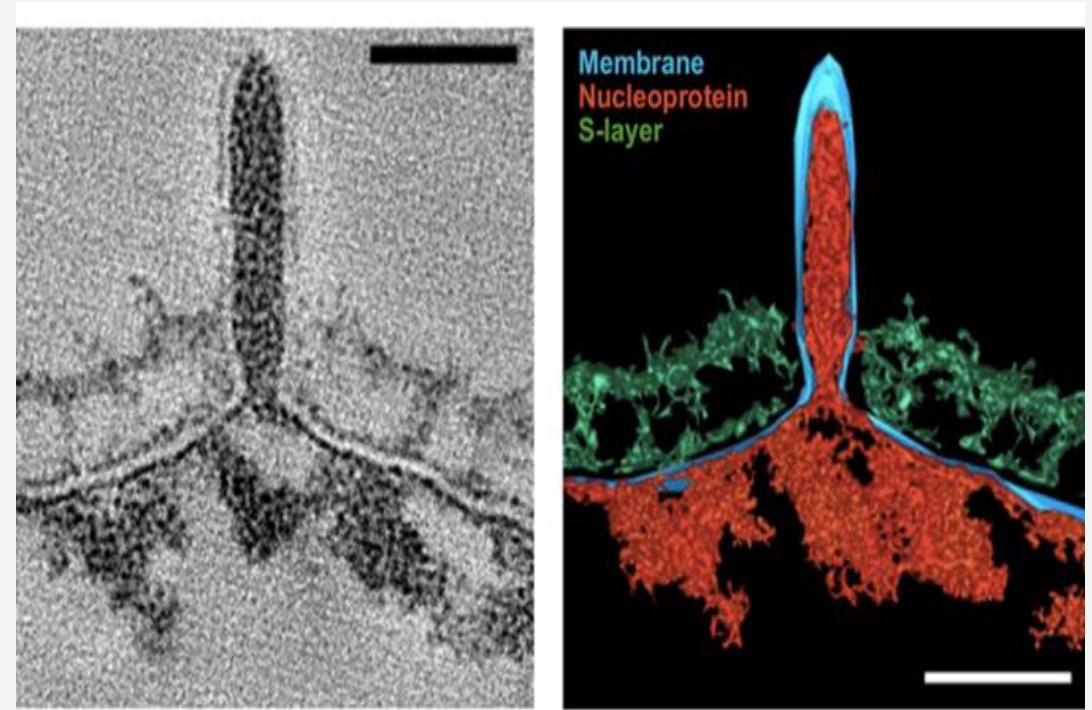
- Host organism - volcanic hot springs
- Cell contains Protein Surface Layers (S-layers)
 - Stability, cell-shape, semi-permeable membrane, etc.
 - Two protein subunits, SlaA and SlaB



Structure of *Sulfolobus Solfataricus* (D.Janckovik/W.Zillig)

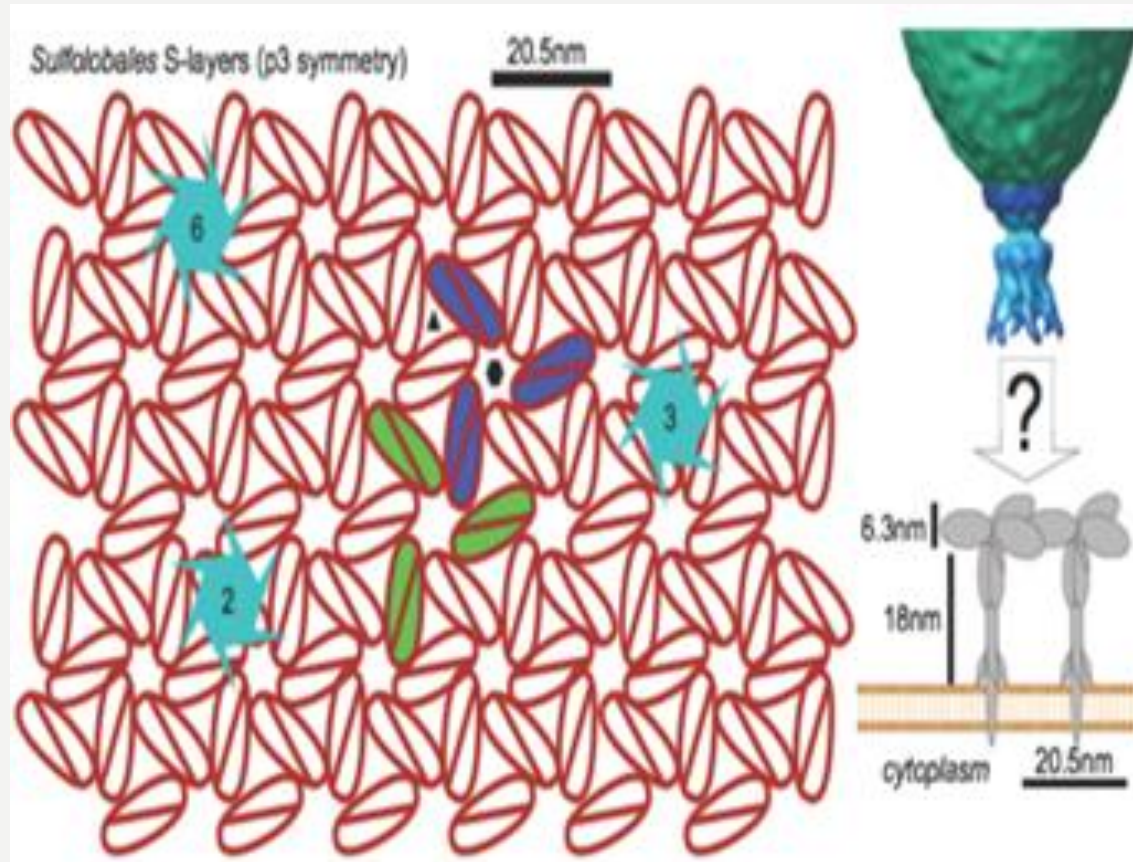
Interaction Between Virus and Host

- Virus-host interaction is not understood
- SSVI capsid proteins localized at membrane surface during egress
- Combining virus with S-layer allows investigation of receptor interaction and infection mechanism

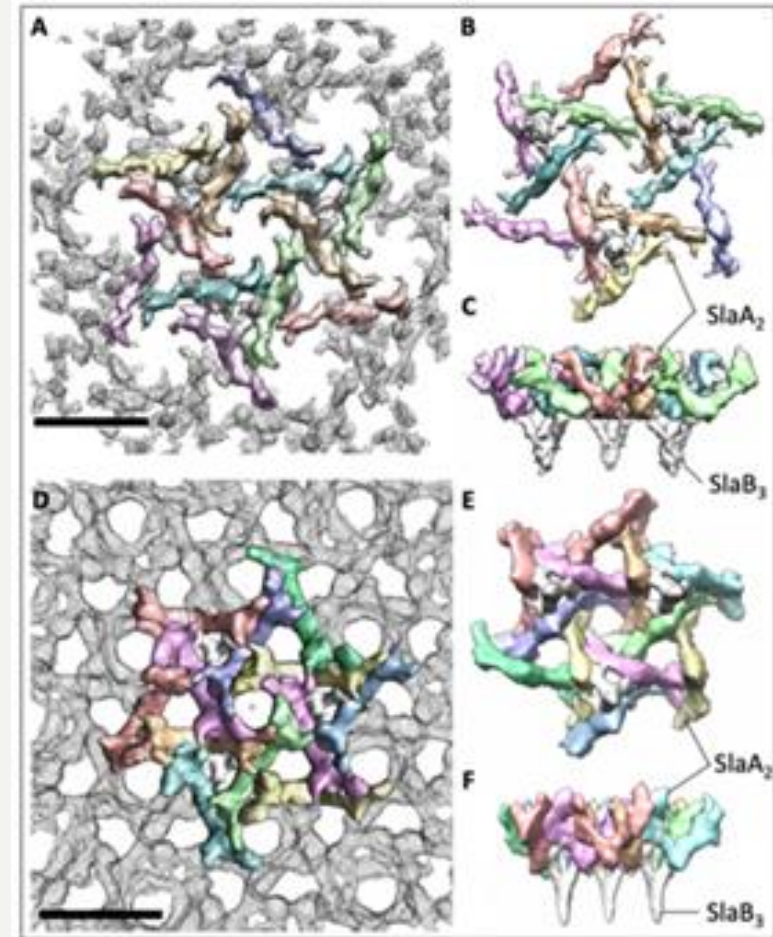


Budding of SSVI from host organism from 2016 Quemin et al.

Expected Results



Model of S-Layer and SSVI Interaction. Stedman Lab

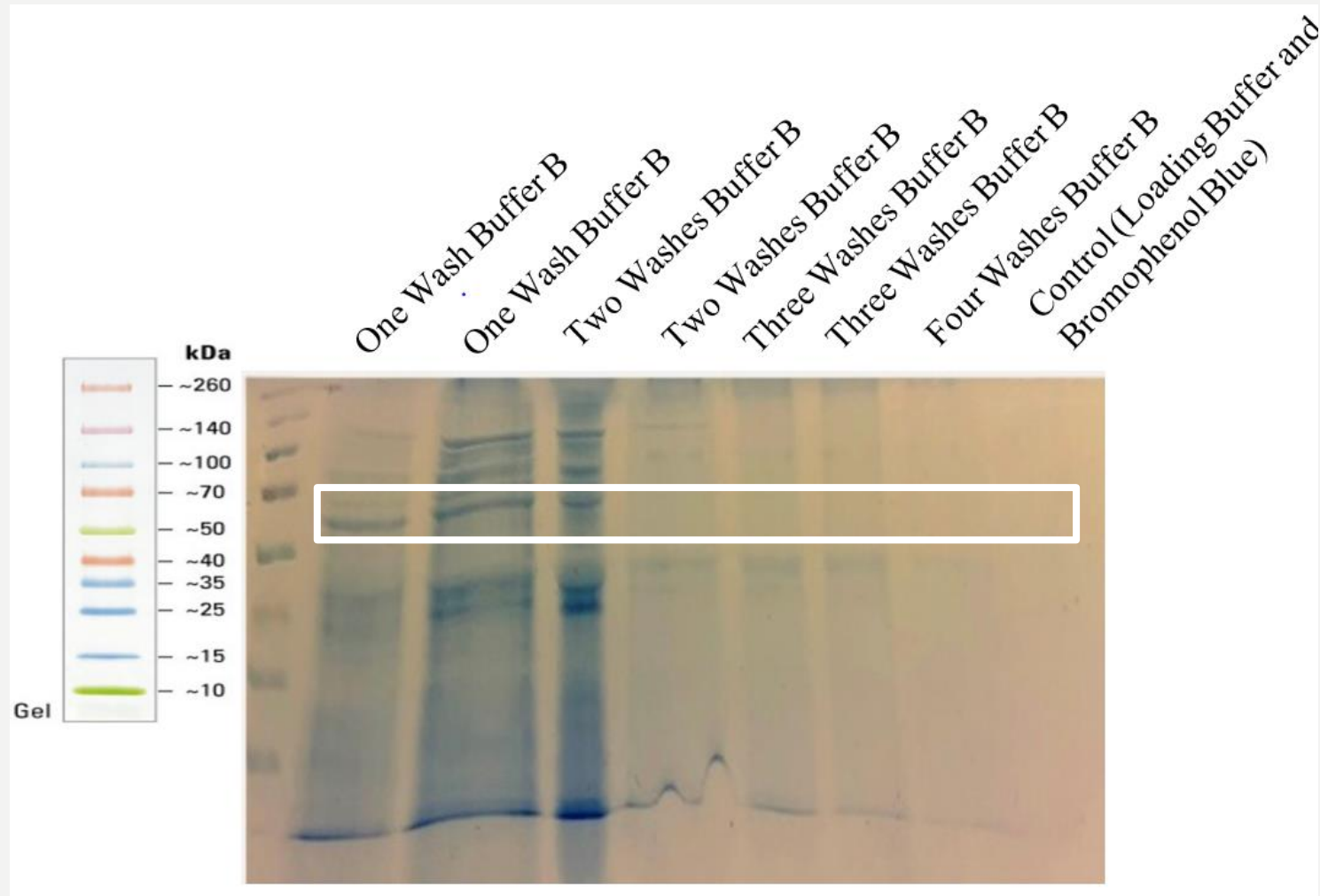


S-Layer Assembly Model. Gambelli et al., 2019.

Methods

- Centrifuge *Sulfolobus* cells after growth
- Freeze and perform multiple washes with two buffers
 - To retain both SlaA and SlaB, only one wash was performed. To remove SlaB, washing with detergent buffer B was repeated an additional three times.
- Visualize samples on 10% polyacrylamide protein gel





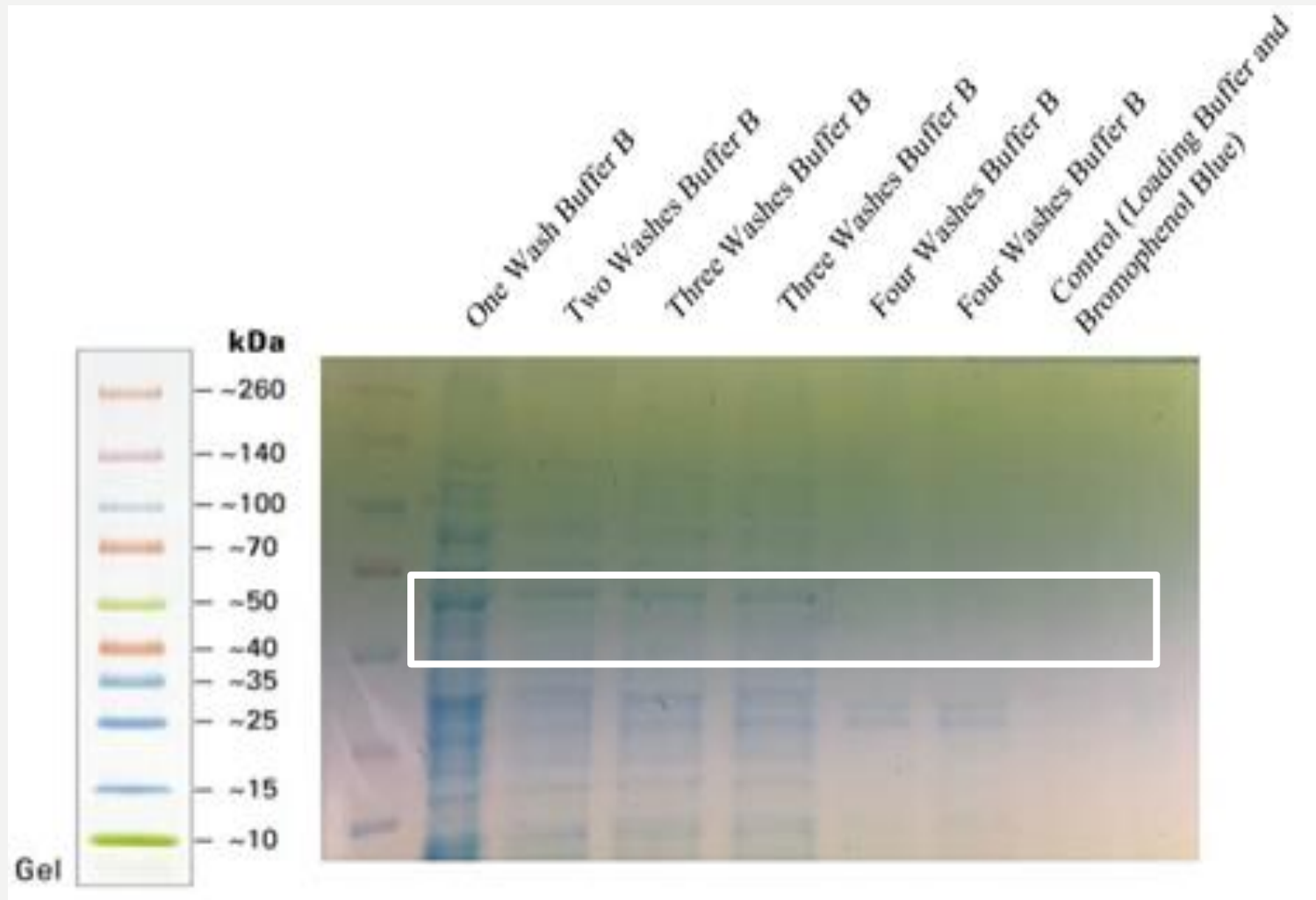
SDS-PAGE of *S. solfataricus* at various stages of purification. All bands were run on a 18% Polyacrylamide Gel. The molecular weight ladder used was the ThermoFisher Scientific Spectra Multicolor Broad Range Protein Ladder.

Complications

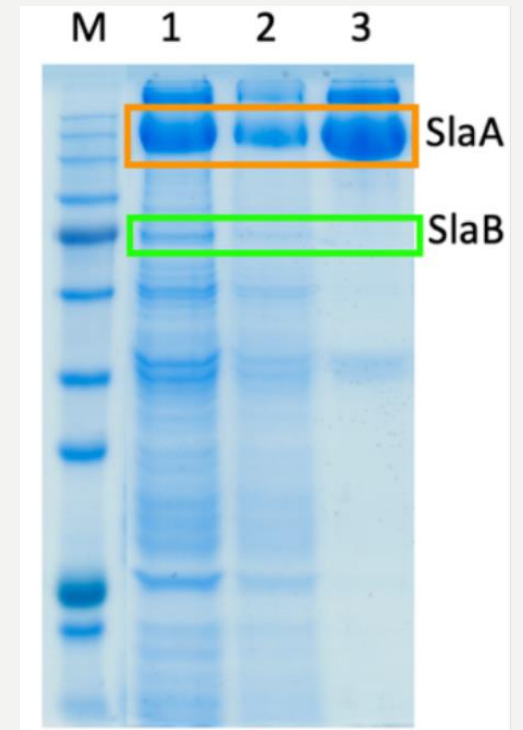
- Slow cell growth
- Lack of bands on protein gels
 - Low concentration
 - Difficulty suspending pellet
- Incorrect % polyacrylamide



Cell incubation at 78 °C



SDS-PAGE of *S. solfataricus* at various stages of purification. All bands were run on a 10% Polyacrylamide Gel. The molecular weight ladder used was the ThermoFisher Scientific Spectra Multicolor Broad Range Protein Ladder



Expected results (Gambelli et al., 2019)

Next Steps

- Image S-Layer with Transmission Electron Microscope
- Add virus, visualize interaction



Transmission Electron Microscope (www.fei.com)

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References

- Gambelli, L. et al.** 2019. Architecture and modular assembly of the Sulfolobus S-layers revealed by electron cryo-tomography. *bioRxiv* 677591. doi:[10.1101/677591](https://doi.org/10.1101/677591)
- Fröls, S., Gordon, P. M. K., Panlilio, M. A., Iverson, E. A., Goodman, D. A., Gorchels, M. E. & Stedman, K. M.** 2017. Extreme Mutation Tolerance: Nearly Half of the Archaeal Fusellovirus Sulfolobus Spindle-Shaped Virus I Genes Are Not Required for Virus Function, Including the Minor Capsid Protein Gene vp3. *J. Virol.* 91.
- Iverson, E. A., Goodman, D. A., Gorchels, M. E. & Stedman, K. M.** 2017. Genetic Analysis of the Major Capsid Protein of the Archaeal Fusellovirus SSVI: Mutational Flexibility and Conformational Change. *Genes (Basel)* 8.
- Iverson, E. & Stedman, K.** 2012. A genetic study of SSVI, the prototypical fusellovirus. *Front Microbiol* 3.
- Quemin, E. R. J. et al.** 2016. Eukaryotic-Like Virus Budding in *Archaea*. *American Society for Microbiology* 7, e01439-16.
- Quemin, E. R. J. et al.** 2015. Sulfolobus Spindle-Shaped Virus I Contains Glycosylated Capsid Proteins, a Cellular Chromatin Protein, and Host-Derived Lipids. *Journal of Virology* 89, 11681–11691.
- Stedman, K. M., DeYoung, M., Saha, M., Sherman, M. B. & Morais, M. C.** 2015. Structural insights into the architecture of the hyperthermophilic Fusellovirus SSVI. *Virology* 474, 105–109.
- Takeoka, A., Takumi, K., Koga, T. & Kawata, T.** 1991. Purification and characterization of S-layer proteins from *Clostridium difficile* GAI 0714. *Journal of General Microbiology* 137, 261–267.
- Zillig, W. et al.** 1980. The Sulfolobus- “Caldariella” group: Taxonomy on the basis of the structure of DNA-dependent RNA polymerases. *Arch. Microbiol.* 125, 259–269.

