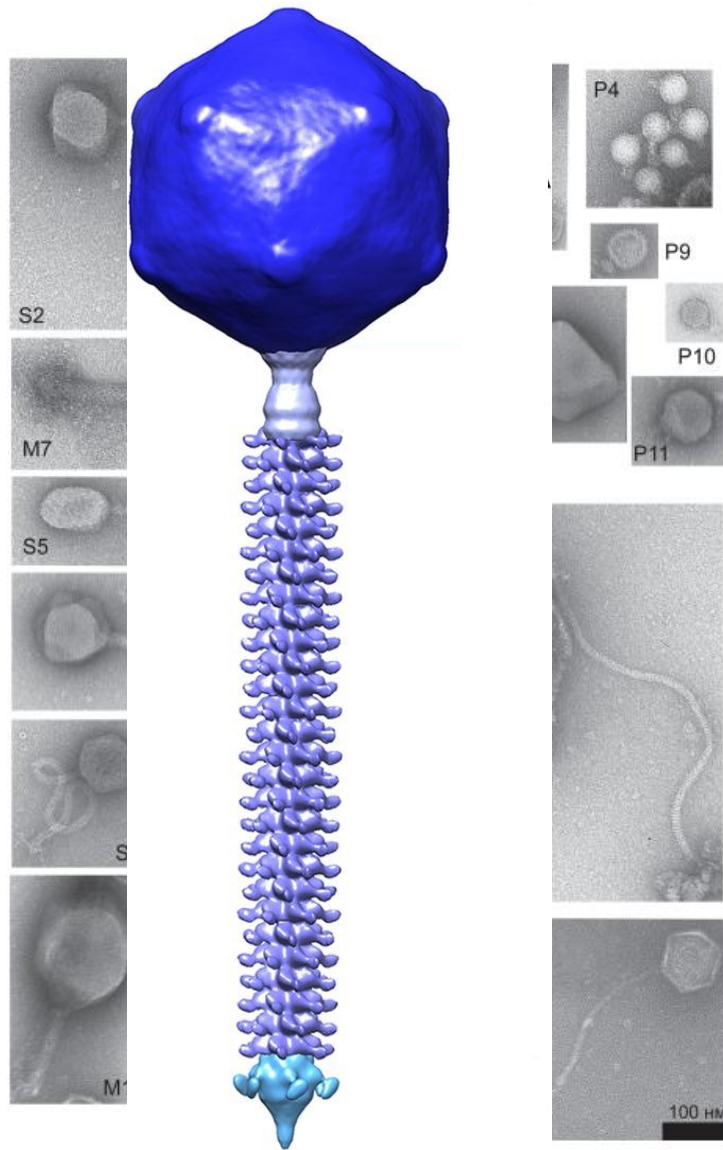


Single-particle Reconstruction of a Prolate Mycobacteriophage Capsid via Negative Stain TEM

Alex Stewart¹ and Claire Rinehart²

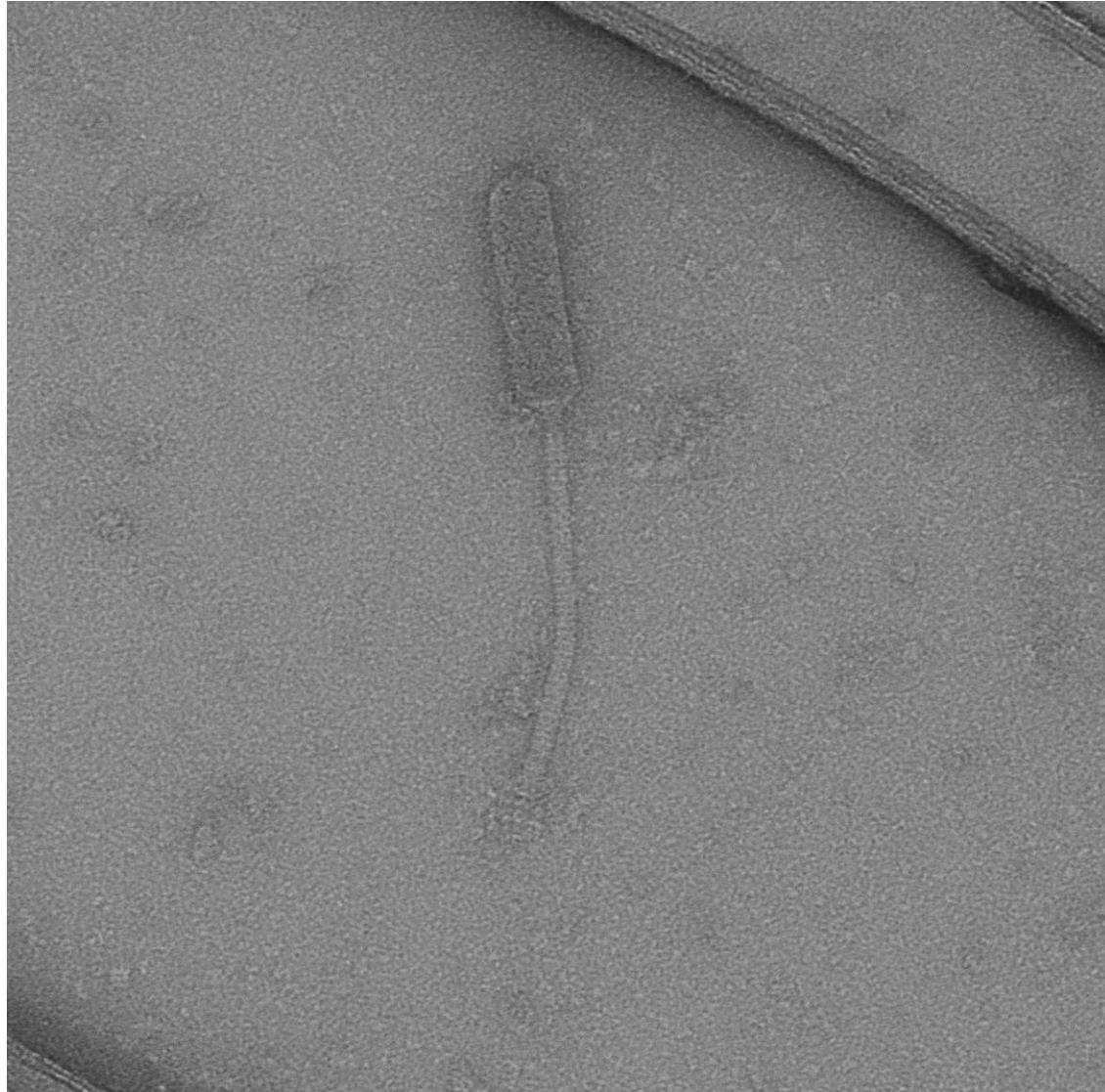
¹University of Kentucky, aast242@uky.edu

²Western Kentucky University, claire.rinehart@wku.edu



What are Bacteriophage?

- Viruses that infect bacteria
- Most have dsDNA genomes
- Most abundant biological entities on the planet
- Vary widely in morphology
- 4 major structural components
 - Capsid (head)
 - Tail
 - Head-to-Tail connector
 - Baseplate

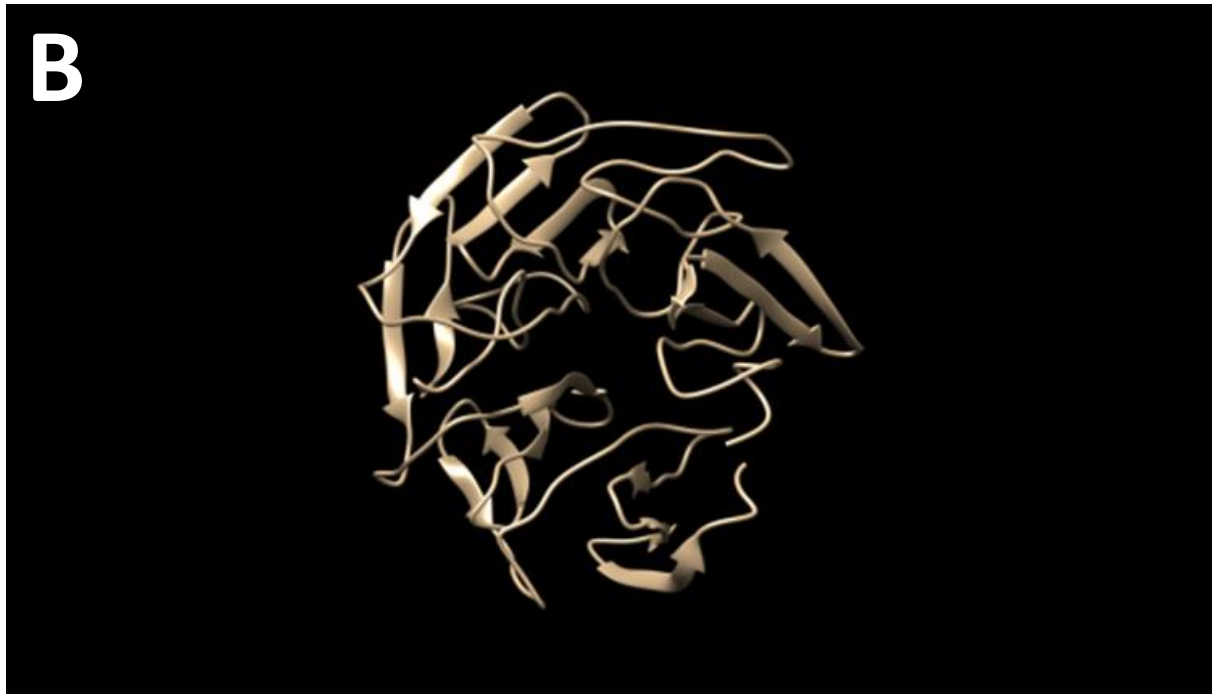


MooMoo

- Isolated from a water sample in Sandy Hook, KY in 2012
- Large, prolate capsid
- Host is *Mycobacterium smegmatis*
- Highly mosaic genome
- ~300nm from the end of the tail to the top of the capsid

MooMoo

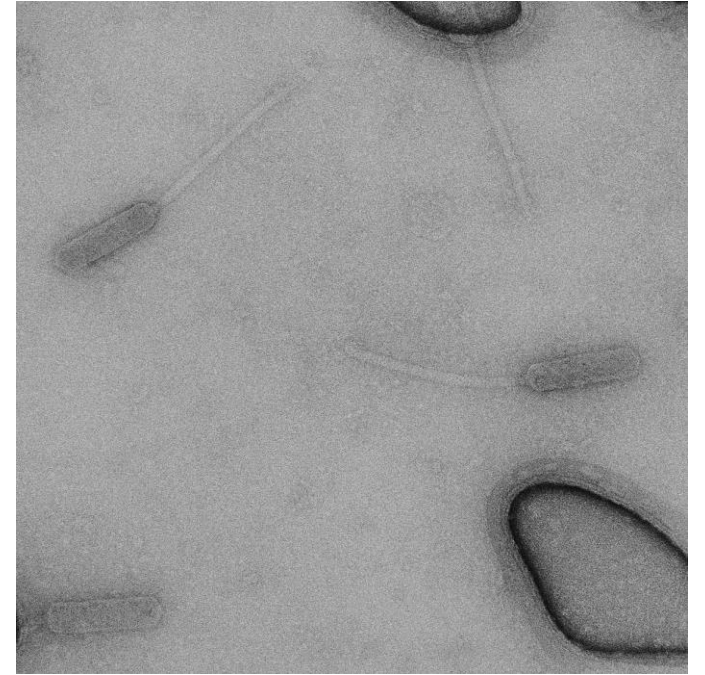
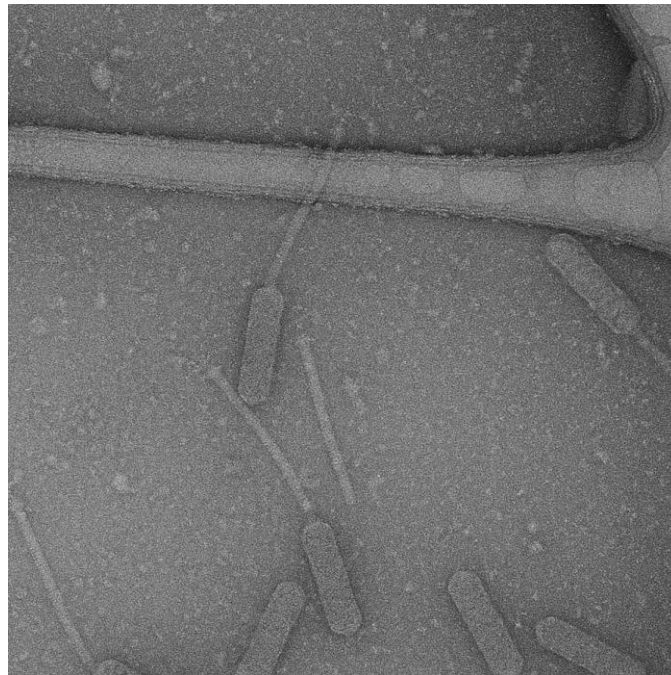
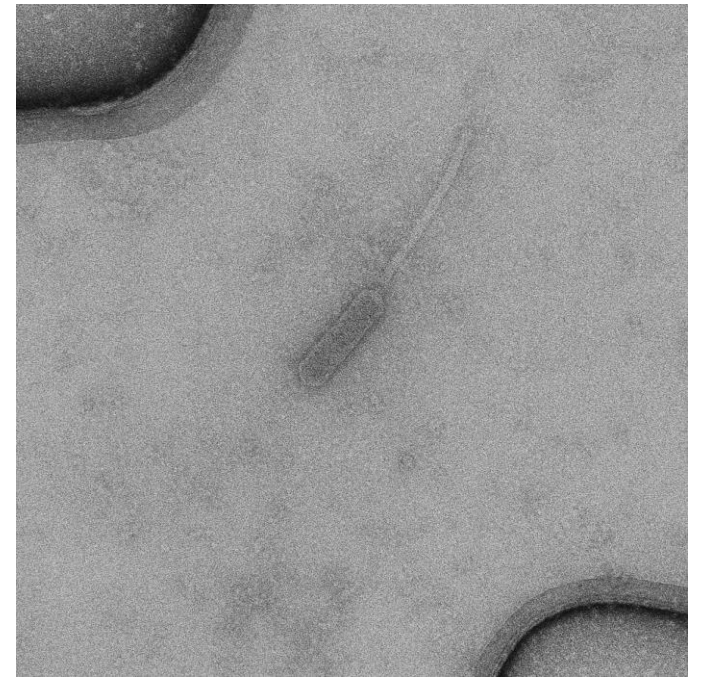
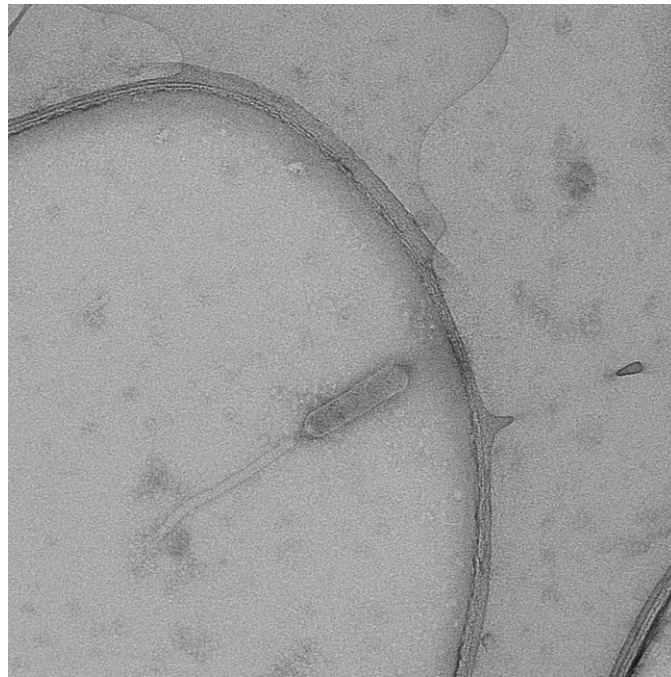
- *In silico* protein folding was previously performed on the major capsid (A) and major tail (B) proteins using I-TASSER
- EM data needed to determine repeat structure and protein orientation within the phage particle



EM Methods

- Automated acquisition of negative stained particles (uranyl acetate)
- 200 kV, 74000x magnification
- Over fourteen thousand images!

Example Images



Data Analysis (ongoing)

- Single-particle reconstruction has been performed using negative stain data for *Araucaria*, another Mycobacteriophage
- We will follow the pipeline established by Sassi et al (2013) using either EMAN2 or RELION software packages
- End result will be an ~ 30 Å reconstruction

Issues Encountered

- Humidity and equipment issues/limitations meant no cryoEM data were obtained
- COVID-related delays
- Data analysis

References

Kulikov EE, Isaeva AS, Rotkina AS, Manykin AA, Letarov AV. Diversity and dynamics of bacteriophages in horse feces. *Mikrobiologiya*. 2007;76:271–278.

Sassi M, Bebeacua C, Drancourt M, Cambillau C. The First Structure of a Mycobacteriophage, the *Mycobacterium abscessus* subsp. *bolletii* Phage Araucaria. *Journal of Virology*. 2013;87(14):8099-8109.

Questions?